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FISH SURVEY EXPERT WORKING GROUP FINAL REPORT

RECOMMENDATIONS FROM CYCLE 1 REVIEW

PREPARED FOR THE
EEM TECHNICAL MANAGEMENT COMMITTEE

EEM/1997/6

Canada

Publications relating to Environmental Effects Monitoring (EEM):

Aquatic Environmental Effects Monitoring Requirements (revised EPS 1/RM/18). Environment Canada. 1997. EEM/1997/1

Pulp and Paper Aquatic Environmental Effects Monitoring Requirements. (revised Annex 1). Environment Canada. 1997. Annex 1 to EEM/1997/1

Tracer Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/2 is also published as: Ali, N et al, 1997. Exposed or not exposed: the effectiveness of using tracers to verify exposure of fish to pulp and paper mill effluent. Canadian Technical Report of Fisheries and Aquatic Sciences 2162.

Dioxin Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/3

Usability of the Resources Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/4

Toxicology Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/5

Fish Survey Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/6

Benthic Invertebrate Community Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/7

Pulp and Paper Technical Guidance for Aquatic Environmental Effects Monitoring (revised). 1997. EEM/1997/8

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Note

The contents of this report are the views of the members of the Environmental Effects Monitoring (EEM) Expert Working Group. As well, the recommendations in this report are the result of the Expert Working Group process and represent the conclusions of the Expert Working Group.

The recommendations and decision trees put forward in this report will be submitted to the EEM Technical Management Committee (TMC). This information will be used by the TMC to produce the TMC recommendations for updates to the regulatory requirements of the EEM program.

The documents which will contain the revised regulatory requirements will be called:

- Aquatic Environmental Effects Monitoring Requirements, EEM/1997/1
- Pulp and Paper Aquatic Environmental Effects Monitoring Requirements, ANNEX 1 to EEM/1997/1

Acknowledgments

The EEM Technical Management Committee (TMC) extends its thanks to the members of the Expert Working Groups for their contribution to the EEM program. The members of the group have produced their reports in a very tight time frame and they have displayed a willingness to work together to meet common objectives.

The review performed by the Expert Working Groups and their recommendations for enhancements to the program are essential ensuring that the EEM program remains based on sound science.

FINAL REPORT OF THE

FISH SURVEY EXPERT WORKING GROUP

FOR EEM CYCLE ONE

AND RECOMMENDATIONS TO THE TECHNICAL MANAGEMENT COMMITTEE

JUNE 27TH, 1997

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1.0 EXECUTIVE SUMMARY

The amendments to the Fisheries Act passed in May, 1992, included a provision for Environmental Effects Monitoring at pulp and paper mills in Canada. The first cycle reports were due on April 1, 1996. An Expert Working Group (EWG) was formed to review the results of the fish survey component, with representatives from government, the pulp and paper industry and consultants with specialized expertise.

The primary objective of the first cycle AFS was to obtain estimates of fish variability in order to allow a proper statistical design in the next cycle. The secondary objectives were to determine the suitability and capture success of sentinel species, to evaluate the suitability of sampling methods and gear, and to assess the adequacy of the reference area. It was not the purpose of the first cycle to link observed differences with the discharge of mill effluents. Therefore, the main focus of the Fish Survey EWG was to review the data from Cycle One as it pertained to the primary and secondary objectives of the first cycle as well as to make recommendations for the second cycle.

The EWG examined the 115 reports submitted. Only 8% of the studies were determined to have been totally successful based on numbers of fish caught, and 53% were unsuccessful . The remainder were partially or marginally successful. The EWG documented a number of generic problems in the Cycle One studies. In marine and estuarine systems, the dominant problems were related to migratory fish and an inability to confirm exposure. No marine and estuarine surveys were totally successful. In freshwater, the dominant problems were related to an inability to catch fish, uncertain exposure, problems with reference sites, the presence of confounding factors and incomplete or poor reporting of data. A review of variability in the measured parameters showed large differences between studies, even those at closely related sites.

It was determined that the generic survey required in the first cycle was not appropriate for all receiving waters, and a decision tree was developed to assist in determining when an alternative approach was warranted. It is felt that the decision tree will be useful for industry, their consultants and Environment Canada's regional coordinators in defining a suitable study design for Cycle Two on a site-specific basis. The decision tree underwent numerous drafts, including consultation with the Technical Management Committee (TMC) and the regions. It was felt that alternative approaches would be required in situations where fish were not present, not exposed, or when the data could not be interpreted. A workshop was held to review the available alternative approaches and their applicability and to recommend research needs. There were no alternatives available for immediate use, but some would be acceptable on a pilot scale.

The EWG evaluated effect sizes, and although consensus was not achieved, government members of the EWG felt that the effect size should be based on a power level of 0.80 and an ability to detect a 20 to 30% difference in gonad size between sites. A series of recommendations were offered for research and development needs for the fish survey component of EEM. This report represents the recommendations of the EWG to the TMC, and may not be fully reflected in subsequent reports and modifications to the regulation.

2.0 INTRODUCTION

The amendments to the Fisheries Act passed in May, 1992, included a provision for Environmental Effects Monitoring at pulp and paper mills in Canada. The objective of the EEM program was to determine if there were environmental concerns at discharge sites in terms of fish, fish habitat or the utilization of the fisheries resource. The first cycle reports were due on April 1, 1996.

The requirements were outlined in Sections 25-38 of the Pulp and Paper Effluent Regulations, a supporting requirements document (AEEMR, 1992a) and in Annex 1 (AEEMR, 1992b) of the requirements document. Additional advice was provided in a Further Guidance document (Further Guidance for the Adult Fish Survey, EEM 1, DFO/EC 1995). The EEM program was designed with requirements to consider effluent toxicity, benthic community structure, and fish utilization (tainting [if there were historical local concerns] and dioxin and furan levels [if chlorine bleaching has ever been used]), and some supporting water and sediment chemistry measurements, as well as a requirement for an adult fish survey (AFS).

Expert working groups (EWGs) have been established to review the first cycle reports. The groups were established for usability of resources (tainting), tracers, dioxin and furan levels, toxicology, habitat (benthic community collections) and the fish survey. The Expert Working Group (EWG) for the adult fish survey (henceforth called the Fish Survey, or FS) component of EEM was selected from government, industry and consulting to review the first cycle reports. The terms of reference were prepared by the EWG and approved by the TMC. Subsequent to the approval of the Terms of Reference, a meeting of Regional Representatives of industry and government prepared a list of issues also to be considered during the Cycle One review. Since the following report prepared by the EWG is a function of the terms of reference, the participants, and the schedule of meetings, these have been included here.

2.1 Objectives of a Fish Survey in EEM

A fish survey component was included in the first cycle of EEM since "*The EEM requirement for pulp and paper mills must directly consider the health and viability of Canada's fisheries if it is to be effective*" (letter from J.C. Crosby, Minister of Fisheries and Oceans to W.M. Vrooman, Chairman of CPPA Environmental Council; May 4, 1992). In terms of the EEM program objectives ("*to assess the adequacy of the national regulations for protecting fish, fish habitat and the use of fisheries resources*"), the fish survey component is used to address the question of adequacy with respect to the protection of fish.

The main purpose of the EWGs was to review the reports as they pertained to the first cycle objectives and to make recommendations for the second cycle. After the development of the regulatory requirements, the primary objective of the first cycle AFS was to obtain estimates on fish variability in order to allow proper statistical design of subsequent cycles. Secondary objectives were to determine a) the suitability and capture success of sentinel finfish species, b) to evaluate gear suitability, and c) to evaluate reference sites.

The Fish Survey EWG has reviewed the objective of the fish survey (to protect fish) and decided that it should be more specific and clearly-defined. Specifically, the purpose of the fish survey component of EEM for future cycles should be to assess the adequacy of the regulations by examining the effects of effluents on the survival, growth and reproduction of fish, where "fish" are defined as finfish, crustaceans and bivalves.

2.2 Concepts and Purpose of Original AFS Approach

The original approach assumed that the growth, reproduction and survival of fish were the most important characteristics to protect. Rather than require extensive and expensive fisheries collections to address this issue, the original AFS approach assumed that a snapshot of information could be used to cost-effectively identify situations where gross problems or fisheries concerns were present. This approach involved sampling adult fish from an exposure area and examining gross indicators of their growth, reproductive performance and age distribution. It was assumed for the purposes of the first cycle that 20 males and 20 females would provide sufficient information to evaluate these responses. It was also decided that two species should be monitored as a minimum and that a minimum of one reference site would be required. Due to concerns about the lack of information in Canadian receiving environments on characteristics of fish performance, it was subsequently decided that the main purpose of the first cycle would be to identify the variability of fish populations so that the subsequent sampling design could be improved on a site-specific basis.

The original interpretive framework of this method was developed from concepts described by Colby & Nepszy (1981) and Colby (1984) and was used by Munkittrick *et al.* (1991) to measure the potential effects of a bleached kraft mill effluent on fish. The method involved the use of one or two adult "sentinel" fish species as indicators of the health of fish populations and has been described in detail by Gibbons & Munkittrick (1994). In brief, fish characteristics were grouped according to age structure (mean age or age distribution), energy expenditure (growth rate, gonad weight, fecundity, age at maturity) and energy storage (condition, liver weight, tissue lipid levels). Based on comparisons with a reference area, a response characteristic for each grouping was assigned as either an increase (+), a decrease (-), or no change (0) for the sentinel specie(s) from the exposure area. The response pattern was then compared against generalized response patterns in order to provide direction and focus for research into the causal factors (Gibbons & Munkittrick, 1994).

In the original AFS approach, 3 to 5 studies (10 to 15 years under the current EEM monitoring requirements) would be required to determine temporal/spatial trends in the data (Hodson et. al, 1996). Given these requirements, the Fish Survey EWG was uncertain whether interpretation would be possible in a period of less than 10 to 15 years. The group further acknowledged that interpretation on a shorter time-frame could require a greater financial commitment. The TMC should evaluate whether this time frame is acceptable for interpretation of these data.

While the measurements included in the original AFS approach have traditionally been used by fisheries biologists to assess the status of exploited populations, there continues to be debate among fishery experts about the appropriate level for monitoring the performance of fish with respect to assessing the effects of contaminants. At the time the original AFS approach was developed, it was considered that measurements on individual fish offered a rough estimation of contaminant effects at the population or community level and that there were no other cost-effective alternatives available. There was some concern expressed in the group that variables measured in the AFS which are not ecologically or biologically relevant to measuring survival, growth or reproduction will continue to be EEM requirements. Specifically, there was uncertainty among some members whether observed differences are indicative of population, community or individual level effects. Other members argued that the parameters being used here have been used successfully as indicators of fish population status by fisheries biologists for more than 100 years.

2.3 Objectives of Cycle One AFS

The primary objective of the Cycle One AFS was to determine the variability in the measured parameters and to use this information in power calculations in order to estimate the minimum sample size requirements for the second cycle. According to the study design requirements, Cycle One could also be used to evaluate the suitability and capture success of sentinel species, the adequacy of reference areas, and the appropriateness of the sampling methods and gear. It was not the purpose of the first cycle to link observed differences with the discharge of mill effluents. As for Cycle Two and beyond, "*...subsequent cycles will be focused on confirmation of differences and the identification of causative factors associated with the differences (if any are present)*" (DFO/EC, 1994). In the view of the industry representatives of the Fish Survey EWG, such a framework encourages mills to undertake research efforts with the purpose of identifying why there are differences rather than understanding how their operations are affecting the fisheries in the receiving environment. Nevertheless, it was acknowledged by the entire Fish Survey EWG that the fish survey component of EEM is not to be used as a means of either developing or validating methods. Industry representatives feel that these activities are clearly within the realm of research, as opposed to monitoring, and are not directed towards addressing the objectives of the fish survey component of the EEM program (*i.e.*, assessing the adequacy of current effluent regulations in protecting fish). Government representatives felt that the parameters have been widely used, are important indicators and are conservative for detecting differences.

2.3.1 Terms of reference

Purpose

The regions, the mills, and the consultants have identified difficulties with some of the EEM requirements provided under the Cycle One monitoring program. To address these concerns, the EEM Technical Management Committee established Expert Working Groups to review the results of Cycle One, identify the technical difficulties and provide recommendations on modifications or alternatives where existing approaches have proven to be unproductive or do not work. These recommendations will provide the basis for Cycle Two requirements.

Objectives

As principal responsibilities, the Fish Survey Expert Working Group (FSEWG):

- reviewed the concept and purpose of the fish survey requirements under the EEM program
- reviewed the AFS data generated in Cycle One and identified situations where the requirements were or were not successfully met
- identified the
    - i. difficulties encountered by the consultants in meeting the program requirements, and provided solutions
    - ii. sampling-related difficulties, and provided alternative approaches
    - iii. QA/QC-related difficulties, and provided alternative approaches
- took into account the situations where the national monitoring approach for Cycle One was proven to be unsuccessful and provided alternative or modified approaches that address these site-specific difficulties, or identified areas requiring further research
- provided a report identifying clear recommendations for Cycle Two requirements

Operating Principles

The work of the FSEWG was based on the following principles:

1. The recommendations were driven by the Cycle One Regional evaluations.
2. A nationally consistent approach was respected while allowing for flexibility in addressing site-specific issues.
3. The recommendations follow the EEM program monitoring criteria and guiding principles as outlined in the General Program Requirements in document EPS1/RM/18.
4. The recommendations will be finalized by June, 1997.

Process

Meetings and teleconferences were scheduled ahead of time but the process allowed flexibility to have meetings when required. The FSEWG was responsible for providing its own secretariat support and structure. Ms. Shelley Luce was retained on contract as the secretariat.

Final Report

A report summarizing the EWG's results and recommendations was prepared and distributed to the EEM Technical Management Committee during the first week of April, 1997. The Final Report was delivered July 12th, 1997.

2.3.2 Participants

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2.3.3 Group's Activities

Meetings were held as follows:

November 6, 1996	co-chairs' meeting
November 20, 1996	teleconference
December 4, 1996	teleconference
December 12, 1996	teleconference
January 17, 1996	meeting and teleconference
January 27, 1997	meeting
February 17-18, 1997	alternatives workshop
February 18-19, 1997	meeting
March 4, 1997	teleconference
March 19, 1997	teleconference
March 25-27, 1997	teleconference
April 29-30, 1997	TMC and co-chairs meeting
June 4-6, 1997	meeting and teleconference
June 20, 1997	comment period closed for final report
July 10, 1997	final TMC meeting
July 12, 1997	final report delivered

3.0 REVIEW OF CYCLE ONE

The primary objective of the Cycle One AFS was to determine the variability in the measured parameters. The secondary objectives were to evaluate the suitability and capture success of sentinel species, the adequacy of the reference area, and the appropriateness of the sampling methods and gear; however, the Fish Survey EWG felt it was important to review a) whether mills were able to catch sufficient number of fish; b) the appropriateness of sampling methods; and c) the adequacy of reference areas, in order to develop a better understanding of the issues and problems in Cycle One. Therefore, the assessment of variability is discussed last in this section. It was the responsibility of the EWG to identify the problems and technical difficulties and to provide recommendations on modifications or alternatives. Since evaluation of whether the objectives were fulfilled is directly affected by problems encountered during Cycle One, the problems will be reviewed first.

3.1 Problem Identification of Cycle One

3.1.1 Marine and Estuarine Receiving Environments

The FSEWG reviewed the Cycle One reports and found a number of problems and confounding factors which were present at mills. Mills which had marine or estuarine discharges faced a number of challenges. In the marine environment the problems included lack of fish, high dilution of effluent (small plume), trapped plumes, migratory sentinel species, unsuccessful tracers, confounding discharges, and problems associated with shipping and dredging (Table 1). The problem associated with trapped plumes requires special consideration. Problems with tracers in marine environments suggest that the degree of exposure of marine sentinel species could not be known or even assumed with the same degree of certainty as that of freshwater sentinel species.

The major constraining factor limiting the success of the Fish Survey was the lack of catch success of the proposed sentinel species. In general, freshwater finfish were more often used as proposed in the study design and had a higher rate of successful catch than marine finfish. Nine of 27 marine mills failed to catch the required number of specimens for even one sentinel species.

Some of the difficulties associated with marine systems relate to the fact that marine systems are generally more open and difficult to sample. Another difficulty is due to the non-homogeneous distribution of habitat and sexes within marine habitats, as evidenced in Dungeness crab, red rock crab and widow rockfish catches in BC. In several instances a preponderance of juveniles (prickly sculpin) were sampled. In some cases life history characteristics of the species tends to segregate the juveniles from the adults in the estuarine exposure zone (e.g. starry flounder). In estuarine environments additional problems were associated with tidal fluctuations and confounding effluents (Table 1).

In an attempt to satisfy the requirements for two sentinel species some mills included invertebrate species, which is permitted within Annex 1. The difficulties associated with this approach relate to factors such as the inability to age crabs and prawns, which are also subject to commercial and recreational harvesting. Other species confound interpretation of reproductive effort (shellfish such as oysters and mussels).

Potential solutions to the difficulties outlined above include a) using alternate species, b) using caged mussels or oysters where such species are appropriate, and c) using mesocosms for confounded situations. Another inclusion for any decision tree (see Section 4.2) was the provision of an exemption in the requirements for a Fish Survey where such a study is impractical for a variety of reasons (no other alternatives possible, etc.).

3.1.2 Freshwater Receiving Environments

In freshwater, receiving systems were divided into river or lake environments. The dominant freshwater problems encountered in Cycle One related to an inability to catch fish and other problems related to fish capture (Table 2). The remaining problems in order of priority were uncertain exposure, problems with reference site selection, problems with confounding effluents, incomplete and poor reporting, and sampling immature fish or fish with undeveloped gonads. Many of these problems were avoidable. For example, capture of immature fish, or of fish with undeveloped or spent gonads, could be avoided by changing sampling gear or timing. In many cases problems associated with too few fish can be avoided by altering sampling strategies and capture techniques, or selecting more abundant species. Some suggestions for dealing with problems with reference sites are discussed in Section 3.2.3.

Table 1. Numbers of mills that encountered problems and confounding factors in Fish Surveys in marine and estuarine receiving environments.

Problems and confounding factors	No. of marine mills	No. of estuarine mills
Total number	7	21
small plume	1	5
remains on surface or on bottom	1	3
extreme tidal fluctuations	0	4
uncertain exposure	9	16
other effluents/historical deposition	1	6
shipping, dredging	1	3
reference sites	2	2

Table 2. Numbers of mills that encountered problems and confounding factors in Fish Surveys in freshwater (river and lake) receiving environments.

Problems and confounding factors	No. of mills - river	No. of mills - lake
Total number	73	10
too few fish	42	7
uncertain exposure	23	2
reference sites	16	1
other effluents	15	3
only one sentinel species	13	3
incomplete/poor reporting	12	3
gonads undeveloped/spent	10	0
fish immature	9	3
physical confounding factors[1]	6	0
aging problems/not aged	6	0

[1] *e.g.* shipping, dredging, fast current, etc.

3.2 Secondary Objectives of Cycle One

3.2.1 Capture Success and Suitability of Sentinel Finfish Species

The information presented in this section on capture success of sentinel species is formatted in order to provide a national and regional summary of the Cycle One results. The information on the suitability of sentinel species is based on the collective knowledge of various members of the Fish Survey EWG.

This review is restricted to finfish, although it should be noted that some coastal mills (which represent 23% of the mills in Canada) did collect crustaceans and bivalves in Cycle One. For these latter species, insufficient information is available, particularly with respect to minimum sampling requirements. Nevertheless, protocols are currently being developed which may prove useful for future evaluation of the Cycle One results and completion of Cycle Two surveys.

3.2.1.1 Capture Success

Classification Scheme

The Fish Survey EWG considered that the Cycle One AFS was "**successful**" if mills were able to collect at least 20 finfish of 2 species for both sexes in the reference and study areas and exposure to the mill effluent could be demonstrated. A Cycle One study would also be considered "successful" if a lack or absence of fish could be definitely attributed to the mill effluent (*e.g.* low dissolved oxygen in effluent plume). However, insufficient information was available to determine if this latter situation occurred at any of the mills. The EWG further considered that a study in the first cycle would be "**partially successful**" if (a) sufficient numbers of one species and about 15 or more (of each sex, at each site) of a second species were collected or (b) sufficient numbers (20 males and 20 females from each site) of only one species were collected.

A third category, "**marginally successful**", was used for mills which generally caught about 15 fish or more of one or two sentinel species and/or where it was apparent that some minimal but additional sampling effort would have yielded the required sample size of 20/20 for both sexes in the reference and exposure areas. It is acknowledged that this classification is somewhat subjective, but the designation of "marginally successful" is considered useful for identifying the number of mills that were almost successful in meeting the minimum sample size requirements of the AFS and may indicate the potential for numbers of successful studies in Cycle Two.

The above definitions were used in the following review of sentinel species capture success, with the exception that exposure to the mill effluent was not a part of the criteria for establishing "success". This is because establishing whether mills were able to catch the required number of fish was of primary interest. For these "successful" mills, the available information was then reviewed to determine if there were any confounding factors or problems, including uncertain exposure to mill effluent See Appendices III and IV).

Most mills (92%) were unable to meet the minimum sample size requirements for 2 sentinel species of both sexes in the reference and exposure areas. On a national basis, only 9% of freshwater mills were successful and 40% were partially successful, while 29% were marginally successful (Table 3). There were no successful surveys at mills which discharge into marine/estuarine systems; 29% were partially successful and 18% were marginally successful (Table 3).

There was considerable regional disparity in the catch success which will affect these values. On a regional basis, the division for both receiving environments is highlighted in Table 4.

Table 3. Canada-wide[1] summary of the sentinel species capture success for finfish in freshwater (FW) and marine and estuarine (M) receiving environments.

Region	Receiving	No. of Studies	Success with 2 species	Partial success (a)	Partial success (b)	Marginal success	No success
Pacific and Yukon	FW	8	2	1	1	1	3
	M	11	0	1	3	0	7
Western and Northern	FW	9	2	4	2	1	0
Ontario	FW	23	2	4	6	7	4
Quebec	FW	42	3	1	5	7	26
	M	5	0	0	0	0	5
Atlantic	FW	5	1	0	1	3	0
	M	12	0	2	2	5	3
Total		115	10	13	20	24	48

[1] one mill excluded from summary because of late submission of their report.

Table 4. Regional success of Cycle 1 fish surveys (as percent of total number of studies) in freshwater (FW) and marine and estuarine (M) receiving environments.

Status of Capture	Pacific and Yukon		Western & Northern	Ontario	Quebec		Atlantic	
	FW	M			FW	M	FW	M
Successful	25	0	22	9	7	0	20	0
Partially	26	36	66	44	14	0	20	34
Marginally	13	0	11	30	17	0	60	42
Unsuccessful	38	64	0	17	62	100	0	25

If the EWG's definition of success was relaxed so that "successful" included mills that captured 15 fish or more of two sentinel species, and "partially successful" included mills that captured 15 fish or more of one sentinel species, capture success results were slightly higher (Table 5). The results indicate that the potential for meeting the minimum sampling requirements for 2 sentinel species in freshwater was highest in the Prairie region. The potential for capturing the minimum number of 1 sentinel species was greatest at inland mills in the Atlantic and Pacific regions and in the Ontario region.

Table 5. Capture success results when "successful" includes mills that captured 15 fish or more of two sentinel species, and "partially successful" includes mills that captured 15 fish or more of one sentinel species (as percent of total number of studies per region).

Status of Capture	Pacific		Prairie	Ontario	Quebec		Atlantic	
	FW	M			FW	M	FW	M
Successful	40	9	63	23	7	0	20	17
Partially	50	27	13	58	26	0	60	42

Regional Synthesis
The information provided to the Fish Survey EWG was synthesized on a regional basis for the various types of successful studies to a) identify which species were used, b) examine whether fish were exposed to mill effluent, and c) determine whether other problems or concerns were identified. The information presented here (Table 6) is detailed in Appendix III and summarized further in Appendix IV.

Pacific and Yukon Region: For mills categorized as successful, partially successful or marginally successful (in terms of capture), a total of 5 species were used in freshwater environments and 3 species were used in the marine environment (Table 6). Of the 13 Pacific and Yukon region mills represented in the above table, at least 4 were unable to confirm exposure to the mill effluent. Other problems that were identified at these mills included small plume/unconfirmed exposure, undeveloped or immature gonads, different habitats, and high numbers of juvenile starry flounder were caught (also see the example provided for Scott Paper and Paperboard in Table 7).

Western and Northern Region: A total of 5 species were used by the Prairie mills where capture was categorized as "partially successful" in Cycle One, with longnose sucker being most common. Of the 6 Prairie and Northern region mills represented in Table 6, at least 2 were unable to confirm exposure to the mill effluent. Other problems identified at these mills included fish migration and difficulty in aging.

Ontario Region: At mills categorized as successful, partially successful or marginally successful (in terms of capture), a total of 12 species were used as sentinels in the Ontario region, with white sucker and longnose sucker being the most common. Of the 21 Ontario mills represented in Table 6, at least 5 were unable to confirm exposure to the mill effluent. Other problems included habitat differences, confounding influences, and undeveloped gonads.

Quebec Region: In the Quebec region, 5 sentinel species were used in the Cycle One AFS at mills categorized as successful, partially successful or marginal successful. White sucker were the most common species. Of the 14 Quebec mills represented in the above table, at least 3 were unable to confirm exposure to the mill effluent. Other problems included confounding influences, aging problems, mobility and undeveloped gonads.

Atlantic Region: There were 7 sentinel species collected in the Atlantic region at mills categorized as successful, partially successful or marginally successful. Of the 9 Atlantic mills represented in Table 6, at least 4 were unable to confirm exposure to the mill effluent. Other problems identified included habitat differences, small effluent plumes and undeveloped gonads.

Table 6. Species used in Cycle One studies demonstrating various degrees of capture success.

Region		Species	No. of Mills
Pacific and Yukon	Freshwater	Largescale sucker	4
		Peamouth chub*	3
		Mountain whitefish	2
		Longnose sucker	1
		Lake whitefish	1
	Marine	Prickly sculpin*	2
		English sole	2
		3-spine stickleback*	1
Prairie and Northern	Freshwater	Longnose sucker	4
		White sucker	2
		Mountain whitefish	2
		Lake chub *	2
		Shorthead redhorse	1
Ontario	Freshwater	White sucker	7
		Longnose sucker	3
		Spottail shiner*	2
		Goldeye	2
		Silver redhorse	1
		Shorthead redhorse	1
		Smallmouth bass	1
		Rock bass	1
		Walleye	1
		Carp	1
		Pumpkinseed	1
		Yellow perch	1
Quebec	Freshwater	White sucker	8
		Longnose sucker	3
		Brown bullhead	3
		Yellow perch	2
		Silver redhorse	1
Atlantic	Marine	Winter flounder	3
		Longhorn sculpin	2
		Tomcod	2
		Cunner	1
	Freshwater	White sucker	2
		Yellow perch	1
		3-spine stickleback*	1

* small-bodied fish

Unresolved Issues

Unresolved issues which require further examination include factors associated with an inability to capture fish in some studies and potential problems associated with interpretation. Examples of each of these concerns follow.

The level of success in Cycle One was disappointing. There may be a variety of reasons why mills in Cycle One were not able to capture sufficient numbers of sentinel species. Examples include:

- unsuitable habitat for AFS
- improper selection of sampling gear or methods
- inadequate fishing effort

All mills in the Western and Northern region had recent field data related to fish surveys and that region had the greatest success (Table 5). Furthermore, mills in other regions where field work was conducted prior to Cycle One surveys also had higher than average success. The EWG recommends that mills where success was limited initiate some focussed surveys prior to conducting Cycle Two work. For new mills or mills which were totally unsuccessful, the EWG may recommend that a reconnaissance survey be conducted prior to design of Cycle Two fish surveys.

There was generally insufficient information available in this review to properly identify the reason(s) for low capture success. Reasons may be difficult to establish, even with a detailed review of Cycle One reports. As an example, an analysis of catch success for prickly sculpin was provided by the Pacific and Yukon region for two mills in the north arm of the Upper Fraser River and showed variability between closely-related sites (Table 7). Both mills have similar habitats (water velocity, mud bottom, tidal environment) and the studies used the same sampling gear. However, the consultant at Scott Paper was unable to catch the required number of prickly sculpin despite a fishing effort more than three times that at Paperboard. The other illuminating observation is that the consultant at Scott Paper, for reasons unknown, did not use peamouth chub or redside shiners as sentinel species. Finally, and demonstrating the importance of site-specificity, the issue of success or lack of success in capture is irrelevant for these mills, given that field surveys may be inappropriate at these locations because of the numerous confounding contaminant sources in the area (A. Colodey, pers. comm.).

Table 7. Summary of prickly sculpin collections at two mills in the Pacific/Yukon region during Cycle One.

	Scott Paper		Paperboard	
	Males	Females	Males	Females
No. in reference	14	6	21	26
No. in exposure	15/4	2/7	22	20
Capture status	unsuccessful		partially successful	
Sampling date	Oct. 17-28, 1994		Nov. 15-Dec16, 1994	
Fishing effort	1150-1610 trap days		64-495 trap days	
Sampling gear	Gee traps		Gee traps	
Other potential sp.	600 peamouth chub 200 redside shiners			

A more thorough review of Cycle One results would be needed to attempt identification of problems and potential interpretation difficulties. For example, fish collected near some sites demonstrated responses in condition which confounded the interpretation of changes in gonad weight. In these cases there may be a conflict in the interpretation of statistical significance when length or body weight were used as a covariate (Table 8).

Table 8. Responses for gonad weight vary with covariate at Red Rock during Cycle One.

Species		Mean		Covariate	p value	Statistical significance
		Reference	Exposure			
White sucker	Male	56.2 ± 10.1	52.4 ± 11.5	FL	0.081	0
		(n = 42)	(n = 29)	CW	0.006	-
	Female	71.9 ± 19.5	66.4 ± 17.8	FL	0.089	0
		(n = 43)	(n = 51)	CW	0.001	-
Longnose sucker	Male	54.4 ± 13.7	76.3 ± 23.0	FL	0.005	+
		(n = 26)	(n = 15)	CW	0.098	0
	Female	97.3 ± 29.3	102.8 ± 45.1	FL	0.008	+
		(n=43)	(n=20)	CW	0.006	+

3.2.1.2 Suitability

According to the AFS Further Guidance Document (DFO/EC, 1995), the selection of appropriate sentinel species will determine the success of the Cycle One fish survey. Munkittrick (1992) described an optimal sentinel species as having an intermediate life span, a fast growth rate, high fecundity, early maturation and being integral in the food chain. The Fish Survey EWG considers that suitable criteria for sentinel species for future cycles are high abundance, reliable exposure and measurable life-history variables, especially for age and gonad weight. Moreover, attention also has to be paid to site-specific situations in evaluating the suitability of sentinel species. For example, lack of barriers may be problematic if mobile species are selected. Moreover, some species are subject to local exploitation and sampling sportsfish may be of public or local concern. Additional problems or concerns identified with sentinel species during Cycle One are highlighted in Tables 9 and 10.

Pacific and Yukon Region

In so far as the suitability of the sentinel species in the Pacific and Yukon region is concerned, largescale suckers and longnose suckers appear to be appropriate species for freshwater (Hatfield Consultants, 1996) . It was also concluded that more information is needed on the movement of all species, particularly mountain whitefish and peamouth chub.

The only apparent problem related to the suitability of sentinel species in the Pacific region is that all starry flounder (3 mills) and some prickly sculpin (1 mill) captured were juveniles. The Fish Survey EWG has concluded that juveniles could be used in exposure areas that are nursery grounds but only for purposes of examining growth; this approach is not satisfactory for evaluating reproduction.

Western and Northern Region

One problem related to the selection of sentinel species in the Prairie region appears to be that the lake chub could not be aged using flatbones or scales; otoliths could have been used (Gibbons, 1997).

Table 9. Species used (in order of frequency) at marine and estuarine mills, and disadvantages or concerns found during the first cycle. There may be additional concerns and disadvantages of which the EWG was not aware. The data in this table include information from both successful and unsuccessful studies.

Species	No. Mills	
Marine mills		
Dungeness crab	4	sexes segregate; aging a problem
English sole	3	geographically separated by sex; adults in deep fjords
winter flounder	2	migratory feeding and spawning; need to be sampled close to spawning
rock crab	2	sexes segregate; aging a problem
Pacific oyster	2	aging a problem; reproduction difficult to evaluate
rock sole	1	
quillback rockfish	1	very old fish captured
Estuarine mills		
winter flounder	5	juveniles; sexes segregated
tomcod	5	migratory
3-spine stickleback	2	difficult to age; gonads difficult to measure (multiple spawners)
longhorn sculpin	2	migrations unknown
Dungeness crab	2	aging a problem
English sole	2	migrations
blue mussel	2	aging a problem, reproduction difficult to evaluate
starry flounder	2	juveniles
prickly sculpin	2	juveniles
rock crab	1	aging a problem
green crab	1	aging a problem
Atlantic silverside	1	migratory
cunner	1	
mummichog	1	multiple spawner
slender sole	1	
widow rockfish	1	very old fish captured (>50 years)

Ontario Region

The only apparent problem associated with the selection of sentinel species in the Ontario region is that the spottail shiners (2 mills) were immature. The Fish Survey EWG has concluded that juveniles could be used in exposure areas that are nursery grounds but only for purposes of examining growth; this approach is not satisfactory for evaluating reproduction. Alternative species should be used if they are available.

Quebec Region

The only apparent problems associated with the suitability of sentinel species in the Quebec region were for mills that were unsuccessful in Cycle One. Either the fish were immature (2 mills) or the habitat was not suitable for a fish survey (2 mills).

Atlantic Region

Although winter flounder were one of the most common sentinels, these species develop gonads within a short time-frame and need to be sampled close to spawning. Use of this species may be further complicated by migrations. In the first cycle, 2 of 3 mills which captured this species found that the gonads were undeveloped. The only other known problems related to selection of sentinel species were that Atlantic silversides were immature (one mill) and that there was a lack of fish diversity (one mill).

Table 10. Species used (in order of frequency) and disadvantages or concerns found at freshwater mills with riverine receiving environments during the first cycle. There may be additional concerns and disadvantages which the EWG was not aware of (total number of mills for this table = 87). The data in this table includes information from both successful and unsuccessful studies.

Species	No. Mills	
white sucker	46	spawning migration
longnose sucker	24	spawning migration
walleye	10	predator, spawning migration, sport fish
yellow perch	7	predator, spawning migration
shorthead redhorse sucker	8	spawning migration
mountain whitefish	5	highly migratory and may skip spawning years
brown bullhead	4	males are guarding young
largescale sucker	4	spawning migration
silver redhorse sucker	4	spawning migration
spottail shiner	4	difficulty getting adults, spawning habits not well-known
carp	2	migration unknown
lake chub	2	otoliths must be used for aging
peamouth chub	2	migration and movements not known
rock bass	2	
burbot	1	predator, migratory
channel catfish	1	predator
fathead minnow	1	
goldeye	1	feeding and spawning migrations
northern pike	1	
threespined stickleback	1	difficult to age; multiple spawner
smallmouth bass	1	predator; game species; mobility
lake whitefish	1	migration
pumpkinseed	1	

The Fish Survey EWG recommended that marine mills should evaluate the use of eelpout, blennies (both live-bearers) and sculpins where available; the best choices for estuarine mills are mummichogs (multiple spawners), killifish and sticklebacks. Multiple spawners and live-bearers require special attention regarding measurement of reproductive variables. The information available for this review does not permit an assessment of whether sufficient numbers can be caught and the EWG recommends that marine sites where the FS was not successful conduct reconnaissance surveys to evaluate the possibility of choosing a different sentinel species.

3.2.2 Appropriateness of Sampling Methods

A further objective of the first cycle was to identify sampling considerations and problems. Since the major problem in the first cycle was collecting sufficient fish, an assessment of some of the problems associated with sampling methodologies was conducted. However, as demonstrated in Tables 7 and 11, capture success in Cycle One varied even at closely-related sites. For example, many of the unsuccessful studies in freshwater environments utilized multi-mesh gill nets (section 3.2.2.2). The EWG was unanimous in suggesting that approaches should be designed site-specifically, and that capture gear and effort be focused on methods shown to be successful.

Table 11. Number of cases (may be more than one per study) using each of the capture methods in marine and freshwater receiving environments. In some cases more than one method is used; the primary method is the one to which greatest effort was devoted or the one which captured the most fish. "Successful cases" are those for which at least 20 fish of each sex were captured.

Primary Capture Method[1]	No. Cases	% Successful Cases (No.)
Hand-picking	5	100 (5)
Seine nets	7	43 (3)
Electrofishing	52	33 (17)
Other (long-lines, handlines, etc.)	3	33 (1)
Gill nets, fixed mesh size[2]	27	22 (6)
Traps or trap-nets	23	22 (5)
Otter trawl	9	22 (2)
Gill nets, variable mesh size	61	15 (9)

[1] complete report not available for 4 studies

[2] 13 cases excluded because mesh size not reported

3.2.2.1. Sampling Considerations for Fish in Marine/Estuarine Surveys

Trawling

The type of trawl gear and sampling strategy used for deployment of this type of fishing gear needs to be carefully considered before surveys are conducted. Unlike many estuaries and coastal regions in Atlantic Canada, the fjords of BC are characterized by steep bathymetry that drops off to hundreds of metres within a few tens of metres of the shore. Diffusers at most of the mills in BC discharge at depths of at least 20 m and so introduce effluent into a steep underwater landscape. In these areas, trawling can be difficult because of the steep angle of the bottom; depth changes rapidly over a few hundred metres of horizontal distance. In these circumstances keeping an otter trawl open using the spreading forces of the otter boards may be difficult because the towing vessel has to maneuver to make quick turns to maintain constant depth. For this reason beam trawls which use wood or metal frames to keep the net open may be preferable. For example, otter trawls were used in fish surveys in Howe Sound, BC and catches of bottom fish were too low to satisfy the basic sampling criteria of the EEM. Part of the reason for this sampling failure may have been due to the use of inappropriate gear and/or attention to some of the problems mentioned above.

Underwater debris such as logs and rocky outcrops also complicate trawling in the fjords and heavy mesh trawls resistant to tearing from snags should be used. The mesh size of the trawl is dependent on the species and size of fish that the survey is targeted on. Because changes in depth and substrate may result in changes in species composition of fish, it is important to ensure that the beginning and ending of trawls are precisely located using differential GPS. This should ensure that the same habitat is sampled in repetitive surveys. Other important variables such as time of start and stop of the trawl, depth, and substrate should also be noted.

Beach seines, fyke nets, and traps
If the effluent is being discharged via shallow water diffusers, the surface effluent field may impinge on shoreline or intertidal fish habitat. In these circumstances, beach seines may be considered as a sampling method. Permanent markers such as small cairns should be set up to mark the beach seine locations. Depending on the steepness of the shore, a small boat may be used to pull the net off the beach, or if the water is shallow it could be pulled by persons wearing chest waders. Beach seines are routinely used for sampling small fish in estuaries and on beaches because they move onto these habitats at high tide. On the Atlantic coast, fyke nets set in the intertidal zone are also an option. Baited traps were also used to catch mummichogs in Atlantic estuaries. Crustaceans such as prawns and shrimp can be caught with a variety of traps. These sampling devices were successfully used in a number of the EEM studies.

Hook and Line and Hand collections
To minimize the impact of fishing gear on habitats, hand collection may be suitable for relatively sedentary species. Mussels were collected by hand in several studies in Pacific region. This type of sampling can be very selective so that only the target species is sampled. For example intertidal blennies and cottids can be collected by searching under rocks and algae at low tide. Rockfish can be collected using SCUBA, a technique which allows sampling to 30m . Using a string of baited hooks (longlining) for bottom fish such as sole should also be considered as these methods are also relatively selective. As with the other techniques precise positioning is vital to make sure the exact habitat can be revisited in the monitoring program.

Gillnets
Gillnets are not recommended for the marine environment because they are usually non-selective and can potentially kill all the sampled fish in addition to the target species. There also can be major logistical problems with this technique because tidal or river currents can cause twisting of the nets. If gillnets are chosen as a technique, they can be sunk to fish at particular depths or floated to sample the surface layers only. Because the pelagic zone in marine receiving environments is typically stratified by salinity and temperature, the fish communities below and above the halocline are usually quite different. If the gillnet is several metres deep, the net will sample both below and above the halocline and the exact habitat where a particular fish species was caught may be difficult to determine.

Purse seines and midwater trawls
Purse seining and midwater trawls may be used to sample fish species that live primarily in the pelagic zone. In order to effectively sample fish from depths where pulp mill effluent may be influencing fish living near bottom or in deeper waters, a fairly deep seine (e.g. 10 m deep) may have to be used. A large vessel such as a commercial salmon or herring seiner may have to be deployed. Midwater trawls have an advantage over seines in that the latter allows more precise layers of the water column to be sampled and in addition the depth that the net is being towed can be recorded and/or monitored electronically and adjustments can be made as needed.

Remote sensing

Video or still camera recording of fish abundance near outfalls can be monitored using cameras mounted on remotely operated vehicles. This technique may be particularly effective in rocky and steep areas where use of fishing gear may be difficult. Camera surveys may also be useful in reconnaissance surveys of bottom conditions before trawls or traps are deployed for fishing.

3.2.2.2 Sampling Considerations for Fish in Freshwater Surveys

Standardized sampling is a priority. Therefore, in situations where sentinel species are the same for Cycle Two as for Cycle One and the sampling techniques were sufficient to capture the target number of each sentinel species in Cycle One, these same sampling techniques should be retained for Cycle Two unless good reasons for change, such as unacceptable by-catch, are documented.

All fish sampling gears are both size- and species-selective. Therefore, choice of sampling gear must be tailored to the sentinel species and the capture environment. Passive capture gear such as trap nets or gill nets may need modification to meet local conditions. If small-bodied fish species are chosen as sentinel species, local capture methods of professional bait fishermen should be considered; frequently their traps are more efficient than those described in the fisheries literature. Capture success at night is generally more successful (for seining or electroshishing) than during daylight hours for most small-bodied species. Local ichthyologists at museums or universities are also good sources for information on capture methods for small species. When large sample sizes are obtained, the method of subsampling should be described and must be statistically valid.

3.2.3. Reference Sites

The selection of appropriate reference sites is essential for the effective interpretation of the EEM Fish Survey results. Reference site selection was identified as a problem at 2 (of 7) marine sites, 2 (of 21) estuarine sites, 16 (of 73) freshwater sites (riverine) and 1 (of 10) lacustrine sites. The specific problems identified were: different habitats in reference and exposure zones, reference and exposure zones close together with no physical barriers between, reference site "inappropriate" or "difficult to evaluate", reference sites on different river systems, and sampling at different depths in the reference and exposed sites.

The tendency of mills to follow the minimal requirement of only a single reference and exposed site makes selection of an appropriate reference site even more critical. Common sense and experience will be the major considerations in achieving success in future cycles. The more familiar the sampling team is with the site and the species being sampled the better will be their ability to select adequate reference sites. It is extremely difficult to separate problems associated with inappropriate selection of sentinel species, sampling gear or effort, from problems with reference site selection. There is no generic method to evaluate or select reference sites, although there are some general principles that should be followed. The EWG recommends that more mills consider increasing numbers of reference and exposed sites where appropriate.

The reference area should be as similar as possible in all respects to the exposed area except for the effluent from the mill. If possible the reference site should be immediately adjacent to or upstream of the exposure zone unless that area is unsuitable (e.g. reservoirs, etc.) and should be sampled at the same or appropriate time period. This requires an intimate knowledge of the physical, chemical and ecological characteristics of the site. The information, knowledge and experience gained in the first cycle of the EEM will be essential in defining the most appropriate reference site in the second cycle. The selection of a reference site in Cycle One should not dictate its use in Cycle Two. If reliable comparisons are to be made then it is critical that the selection of the reference site(s) is appropriate. The selection process must consider the physical, chemical and ecological characteristics of the exposed site as well as the biology of the sentinel species.

The biology and life history of the sentinel species must be as well-characterized as possible to facilitate the prioritization of the site-selection criteria. The ability of the species to meet the objectives of the EEM should be the major consideration, rather than commercial or sport significance of the species. The mobility of the species will help to define the habitat characteristics and may eliminate adjacent or immediate upstream sites as reference sites. For example, a small fish such as a sculpin may have a relatively small home range (meters) while the home range of a predatory fish such as a walleye may be quite large (tens or hundreds of kilometers). The fish may however, range over large areas during different seasons or life stages, especially during spawning, while remaining relatively sedentary over the rest of the year. The timing of sampling is therefore critical and reference sites may be inappropriate during some seasons while ideal in others. The selection of sentinel species is discussed in a different section but is intimately tied to the selection of appropriate reference sites. Factors such as mobility, habitat preference, home range, feeding strategy, etc. need to be considered when selecting a reference site for each species.

There are very few sites where an adequate reference site will not be available. However, in several locations an alternate approach would be preferred, or in a very few cases fish collections may be inappropriate and an alternate may be required. At least 17 freshwater studies used reference sites where an adequate number of fish from one or both sexes were not collected. There were also numerous sites where adequate sample sizes were not collected at either the exposed or reference sites. It is difficult to determine if this was a result of poor site selection, species selection, sampling season or fishing effort.

It should be recognized that selection of an inappropriate exposure site is also possible. At least 16 freshwater mills did not collect adequate sample sizes, of one or both sexes, of the sentinel species at the exposed site even though the species was collected at the reference site. This suggests several serious problems. If the sentinel species (usually a second species) is not available in the exposure zone then an alternative species must be selected. A sampling strategy for each species must be developed, which may require a different sampling approach including use of different exposed sites (still within the exposure zone) or reference sites for each species. The sentinel species selection must be determined by the species present in the exposed area. There is no purpose in collecting fish from a reference area when that species was not available in sufficient numbers in the exposed area. This represents a need to redesign the survey for subsequent cycles. The selection criteria for the exposed site should not be so rigid as to prevent the collection of fish or selection of an adequate reference site. The exposure site data are of little use if an adequate reference site is not available, and the reference data are of no use if no data on fish from the exposure area are available.

In the first cycle there were several examples of studies that selected inappropriate reference sites (reservoirs, mobility of fish, etc.). Some sites immediately adjacent to the mill (e.g. upstream in rivers) may be unsuitable because of dams, natural habitat differences, etc. Although dams provide a barrier between the exposed and unexposed fish they may also cause significant habitat changes, including depth, flow, temperature and structural differences which can alter the performance of some fish species. The physical barrier may preclude species from the upstream areas by limiting habitat or spawning environments, etc., leading to differences in inter-species competition and limitation of food and habitat. Many fish species are extremely mobile and care must be taken to ensure that the reference site is not influenced by the effluent even though it is upstream or on an adjacent system (e.g. Northern Rivers Basin Study, some Athabasca River Cycle One studies). Again the quality of the reference site is dependent upon the sentinel species selected. In many cases the reference site nearest the mill is obviously inappropriate and alternative sites need to be selected or added to the sampling strategy.

There are numerous confounding factors which can affect the selection of reference sites such as effects of other municipal or industrial outfalls (e.g., Fraser River, Saint John, Port Hawkesbury, etc.). Whenever possible, the effects of these factors should be incorporated into the reference site selection; however, in many cases there will not be a suitable site which is influenced by the pulp mill effluent alone and an alternative species or approach would be preferred. There are several mills in Canada which have a municipal outfall adjacent to the pulp mill outfall (e.g. Espanola) or even using the same discharge pipe. Depending on relative volumes, the discharge should not be expected to have no other stressors, but the mill should be a dominant factor. Other sites are confounded by CSOs (e.g. Thorold), or other non-point sources of pollution. Evaluating or controlling for historical damage is also difficult. Although this damage may be due to past effluent discharge from the mill it may also be historical damage caused by other outfalls. There may also have been historical habitat damage caused by related activities, such as log booms, etc. It is not necessary to have a site free from historical damage, nor should the presence of confounding factors automatically cause a rejection of the Fish Survey potential. The situation should be evaluated on a site-specific basis to determine the best approach to meet the EEM objectives.

The more information available from each cycle the easier it will be to interpret the results and to design future cycles. The physical, chemical and biological habitat of the exposure and reference sites should therefore be described in as much detail as possible. As a minimum, this should include measurements of temperature, depth, flow, etc. and a description of the habitat which was sampled.

There is considerable natural variability in the parameters that are being measured in the Fish Survey. This is a result of many factors including differences in habitat, food availability, competition, and physical and chemical environments. It is critical that comparisons to exposed sites be made with consideration of this natural variability. The possibility of using a general data set of regional reference sites has been proposed but this would have to be approached carefully. It would be extremely difficult to match the receiving water characteristics of several mills within a region. Any differences in the timing of sampling could have dramatic effects on interpretation. Although there may be exceptions, the regional approach would generally require more reference locations than the traditional approach. It would however, strengthen interpretation of data if several reference sites within a region were used to determine the general variability in the parameters being measured. The actual comparisons would have to be limited to sites that are known to have very similar characteristics (*i.e.* the reference sites). This approach is therefore prone to problems and does not lead to greater understanding than an approach of simply having multiple reference sites. In some situations such as the Fraser River, Athabasca River, Moose River, and Peace River, there may be opportunities to conduct joint studies which may include reference data collected for more than one mill. This could greatly enhance the interpretation of the data as long as the data is collected in a consistent manner (same gear, season, etc.) and site-specific reference sites are still included for each mill.

In the context of the Fish Survey, there is no strong rationale for having a single sample site for benthos and fish sampling. The characteristics of the fish species selected, the mobility of the fish, different habitat selection and the type of sampling gear required may make the optimal benthos sampling sites inappropriate for the Fish Survey. Although the sites may be the same in many circumstances this should not be a significant criteria for fish sample collection; however, when possible there are advantages to selecting similar sites so that the benthic data can be used to help interpret differences in fish populations.

3.3. Primary Objective of Cycle One – Fish Variability Analysis

Variability was site- and species-specific and was affected by capture gear. The discussion of variability analysis includes a discussion of ecological significance and target effect sizes and a detailed discussion of estimates of variability from Cycle One for white sucker. Variability data for other commonly used sentinel species are also presented (Table 13).

3.3.1. Ecological Significance and Target Effect Size

This section discusses the ecological significance of a Fish Survey and the ecological significance of the target effect size proposed by the EWG (Section 1.2, Appendix II). A Fish Survey is defined as the specific type of survey conducted in Cycle One. The generic term "fish survey" refers to any type of survey which could be used to measure effects on fish, and includes alternatives discussed by the EWG. Although the discussion of ecological significance, and especially target effect sizes, focuses specifically on a typical fish survey, many of the points made are applicable to alternatives.

Ecological Significance
As defined in Annex 1 (EC/DFO, 1992) and in the AFS Further Guidance Document (EC/DFO, 1995), the Cycle One Fish Survey consisted of a field survey of demographic

variables (age structure, growth, reproductive effort) measured on adults of two sentinel species, collected from reference and exposed areas. The key features of the Fish Survey were that adult fish or shellfish were used as sentinel organisms, that demographic variables were used as response variables, and that the survey or effects assessment was conducted in the field rather than in the laboratory. These features must be ecologically relevant, and absent from other EEM program elements (laboratory toxicity tests; invertebrate community survey), for the Fish Survey to be ecologically significant and make a unique contribution to the EEM program.

The *Federal Fisheries Act* protects fish (finfish and shellfish), fish habitat, and the utilization of fisheries. The Invertebrate Community Survey addresses the issue of whether current regulations protect fish habitat. However, except for laboratory toxicity tests on early life stages, the Fish Survey is the only element of the EEM program which assesses potential impacts on fish. Regulatory significance does not always equal ecological significance. However, fish are also important members of aquatic communities, occupying intermediate and top trophic levels. In contrast, benthic invertebrates occupy lower trophic levels. Therefore, an EEM program without a fish survey would ignore an ecologically important part of the aquatic community.

The Fish Survey focuses on adult fish. For practical reasons, laboratory toxicity tests used in the EEM program are conducted on only a small portion of the life cycle, usually early life stages. The traditional justification for conducting early life stage tests is that survival and growth of early life stages are the most sensitive endpoints (e.g., McKim, 1985). However, reproduction (fecundity) may be the most sensitive endpoint, and the most important variable determining contaminant effects on fish population abundance (Barnthouse et al., 1987, 1989, 1990; Suter et al., 1987). Reproductive effects can only be measured on adult fish, but if no adult fish are available, information on growth can be obtained from juvenile fish.

Fisheries biologists often use variables such as those measured in an EEM Fish Survey to assess the status of fish populations and predict future changes in abundance (Ricker, 1975; Getz and Haight, 1989; Smith, 1994). Abundance and biomass, and their changes over time, depend on age- or stage-specific survival (or its inverse, mortality), growth, and fecundity. Growth and fecundity are directly measured in an EEM Fish Survey. Age structure acts as a qualitative surrogate for more direct measures of effects on survival which usually require estimates of abundances.

Laboratory toxicity tests have two major disadvantages relative to the Fish Survey and other types of field survey. The laboratory environment is unrealistic, and results of laboratory toxicity tests are not easily extrapolated to field populations. Furthermore, toxicity tests, or at least those used in the EEM program, cannot assess long-term effects, especially on adult fish. Therefore, a comprehensive and effective monitoring program should include both laboratory toxicity tests, and a Fish Survey or other field survey.

Barnthouse et al. (1990) examined the uncertainties associated with extrapolating laboratory toxicity test results to field populations, and concluded that:

> "the quantitative effects of both factors [differences in life history between species; exploitation intensity] are negligible compared to the uncertainty introduced by estimating long-term effects from short-term tests or QSARs."

Therefore, what is the advantage of controlling confounding factors in the laboratory, when simply conducting the test in the laboratory is the major source of uncertainty? If the major source of uncertainty in assessing population-level effects is associated with extrapolating laboratory results to the field, then the obvious solution is to measure the important variables directly in the field. The important variables are survival and/or age structure, growth and reproduction (Barnthouse et al., 1987, 1989, 1990).

Target Effect Size(s)

The primary objective of the Cycle One Fish Survey was to obtain estimates of the variability in the demographic parameters measured (EC/DFO, 1995). These estimates of variability would then be used to calculate the sample sizes required in Cycle Two and subsequent cycles to detect ecologically significant effects. Obviously, an ecologically significant effect must be defined before required sample sizes can be calculated.

In the absence of any strong guidance on the magnitude of ecologically significant effect sizes from the literature, the FS EWG proposed that a ±25% difference in gonad weight relative to body weight or length (=relative gonad weight) between reference and exposed areas be adopted as a target effect size. The proposed effect size was developed partly to expedite evaluation of Cycle One results and statistical power calculations for Cycle Two. The major advantages of the proposed target effect size are that it is based on the most important/sensitive variable demonstrated in pulp mill studies (reproduction) and it is probably superior to most alternatives.

Reproduction is probably the key parameter for monitoring pulp mill impacts in the Fish Survey (Section 1.1, Appendix II). Effects of pulp mill effluents on reproduction have previously been measured in field surveys (Sodergren 1989; Munkittrick *et al* 1991, 1992, 1994) and in full- and partial life-cycle toxicity laboratory toxicity tests (Robinson 1994; Kovacs *et al* 1995, 1996). These studies also showed that reproductive and other effects were absent below some mills, or only occurred at higher concentrations of some effluents, suggesting that site-specific factors (dilution, process, treatment) affect the responses in field situations. There is a need to determine the current extent of reproductive effects in Cycle Two, given recent improvements in process and treatment since the cited studies were conducted.

The 25% difference chosen for the target effect size (ES) should be regarded as approximate; a range (i.e., 20-30%) would be more appropriate. Excessive precision should be avoided when specifying target ES and calculating sample sizes for the Second and subsequent cycles (Section 3.3.2). The lower limits for the target ES were set by two considerations:

(1) Many AFS would not have sufficient statistical power to detect smaller effects (e.g., <20% and certainly <10%), except over many cycles and/or with larger sample sizes than in Cycle One. As target ES become smaller, and required sample sizes larger, the effects of sampling mortality on the population will eventually exceed any population-level effects from reproductive effects.

(2) Differences in relative gonad weight of <20% may occur naturally between unexposed and relatively similar areas (Appendix II). If the target ES is too small, then the risks of false positives (detecting a natural difference between areas and attributing it to the mill effluent) increase.

An upper limit for the target effect size was more difficult to define, although a reduction of 100% (i.e., reproductive failure for exposed fish) is obviously unacceptable. The proposed effect size of 25% was chosen partly because it corresponds with the effect size observed at several Canadian mills and is within the range of differences observed at several mills in Cycle One (Table 12). Swedish studies have identified larger effects from past pulp mill discharges, although recent studies have indicated that reproductive consequences continue to be a concern (O. Sandstrom, pers. comm.). For some mills, treatment and process improvements made in response to new regulations and public concerns reduced reproductive effects from ≥25% to <25%. Therefore, it is reasonable to expect effect sizes of less than 25% below modernized mills adhering to current regulations. If one chooses a higher target effect size (e.g., 50%), then it becomes more difficult to evaluate the effectiveness of the regulations (the objective of the EEM program), because effect sizes below many mills prior to recent improvements may already have been <50%.

Although reproductive effort (relative gonad weight) is arguably the most important variable in the Fish Survey, the EWG recommended that effect size be set or calculated for other variables. These effect sizes could be set in the same way as those for relative gonad weight - greater than natural differences but similar to effects observed in Canadian and Swedish studies. Alternatively, sample sizes could be set based on the target effect size for reproduction, and effect sizes which could be detected for other parameters calculated and reported. These detectable effect sizes for other parameters should still be compared to natural differences effects observed in other studies as part of interpretation or even study design for Cycle two (Section 3.3.2).

Table 12. Percent differences in female gonad weights at body weight, where differences were statistically significant. "% Difference" is for exposed relative to reference sites.

Mill	Species	Reference (n)	Log$_{weight}$ reference	Antilog	Exposed (n)	Log$_{weight}$ exposed	Antilog	% Difference
Gatineau PQ	WHS	17	4.6832	108.1155	21	4.0616	58.0671	-46.29
La Tuque PQ	WHS	17	4.7303	113.3296	12	4.1605	64.1036	-43.44[2]
Napanee ON	RB	21	-0.4763	0.3340	23	-0.7541	0.1762	-47.25[2]
Marathon ON	LNS	20	1.7603	57.5771	20	1.5800	38.0216	-33.96[2]
Pine Falls MB	WHS	23	1.7490	56.1048	28	1.6092	40.6631	-27.52
Thunder Bay ON	WHS	20	1.8011	63.2616	20	1.6617	45.8870	-27.46
SS Marie ON	YP	26	0.9028	7.9945	27	0.9857	9.6756	+21.03
Dryden ON	WHS	20	1.9347	86.0340	20	2.0188	104.4191	+21.37
Dryden ON	Wall	17	1.7595	57.4818	20	1.8942	78.3718	+36.34
Portneuf PQ	WHS	15	1.7918	61.9142	21	2.2508	178.1517	+187.74[2]

[1] In 10 studies, relative gonad weight was decreased in exposed fish , but adjusted means not reported or the complete report was not available. In 6 studies, relative gonad weight was increased in exposed fish , but adjusted means were not reported or the complete report was not available.

[2] Gonad weight relative to age rather than body weight.

3.3.2. Variability of Fish Survey Parameters

Estimates of Variance from Cycle One

Table 13 summarizes variances of the AFS parameters from Cycle One for white sucker, the most commonly used sentinel species. These variances are expressed as the antilog of the square roots of Mean Square Errors (MSE) from ANOVA analyzing log-transformed ages, and from the ANCOVA analyzing log-transformed regressions for other parameters. To obtain approximate Coefficients of Variation (CV) for untransformed or arithmetic values, CV (in %) = 100 × ({antilog √MSE} - 1). The Further Guidance Document (EC/DFO (1995; pp. 28-29) provides a good summary of the use and interpretation of variances of log-transformed values. Table 13 includes only those studies which reported MSE for log-transformed data from ANOVA or ANCOVA.

Variances for reproduction (either relative gonad weight or fecundity) are greater than those for growth in length and condition, and similar to those for age, growth in weight, and relative liver weight (Table 13). Differences between the sexes within the same study were relatively small compared to differences between studies. Ranges of variance for any parameter among studies within any species were broad, suggesting that variances could be reduced in many studies and/or that the most suitable species, in terms of statistical power, may be site-specific.

Variances for most parameters were generally lowest for white sucker, but again, the differences among species were relatively small compared to the ranges of variances within species (i.e., among studies). Similar data is also presented for other, commonly-used species.

Power Analyses for Cycle Two FS

Figure 1 provides the sample sizes necessary to detect target ES of 20, 30 and 50% changes in relative gonad weight for the range of variances observed for relative gonad weight in Cycle One. Variances were given as CV for simplicity, but similar power curves would apply to variances given as antilog RMSE for log-transformed data (i.e., CV ≈ 100 × {antilog RMSE - 1}). Note that required *n* increases exponentially with increasing variance, which is a key relationship for Cycle Two FS. Required sample sizes are also much greater for smaller ES, especially when variances (CV) are large.

Appendix II provides extended statistical power analyses for interested readers. To summarize those results, many Cycle One studies, even some which were unsuccessful in terms of achieving minimum sample size requirements, will have sufficient power to detect the target ES of a 25% change in relative gonad weight if repeated in Cycle Two. In these studies, the key consideration will probably be to establish that the areas compared differ in exposure to the mill effluent. Other Cycle One AFS with high variances for reproductive and other variables may not have adequate power. Only limited gains in power can be achieved in these studies by increasing sample sizes within cycles to *n*>20 or increasing the number of cycles. Instead, these studies should focus on reducing variances; if that fails, alternatives to an AFS should be considered. Appendix II provides a limited discussion of methods of reducing variances in AFS. Future EEM guidance documents should explore the factors affecting variance of the AFS variables more thoroughly, and suggest other means of reducing those variances.

There are real risks associated with calculating sample sizes required to detect any precisely defined ES. Sample size (*n*) is very sensitive to small changes in the other power variables (ES, CV or SD, *P* or power). To illustrate the dangers of excessive precision, consider a hypothetical case in which an investigator has surveyed two species in Cycle One (n=20 for each), one with CV=20% for GSI and the other with CV=30%. These two estimates of CV are not significantly different at p=0.05 or even at p=0.20. The investigator should have little confidence that the same species differences in CV will exist in future cycles, especially

Table 13. Summary of antilog RMSE from AN(C)OVA analyzing log-transformed AFS variables for the most commonly-used sentinel species.

Notes: BW=body weight-at-age, L=length-at-age (standard or total), Cond=condition (body weight -at-length), LW=liver weight-at-body weight or length, GW=gonad weight-at-body weight, length, or age, Fecund=fecundity-at-body weight, length, or age. "Method" is the primary fishing method used. 1=single-sized gill nets, 2=multi-sized gill nets, 3=electroshocking, 4=seining, 5=traps or trap nets, 6=hand-picking, 7=other (longlines, handlines, etc.) and 8=bottom trawl.

A. Female white sucker.

Mill	Receiving	Dates	Method	Number Reference	Number Exposed	antilog RMSE age	antilog RMSE BW	antilog RMSE L	antilog RMSE Cond	antilog RMSE LW	antilog RMSE GW	antilog RMSE Fecund
Kenora ON	river	22-29 Sep	2	25	23	1.3944	nr	1.0459	nr	1.2197	1.2233	1.1812
Laurentide ON	river	30Sep-7Oct	2	8	8	nr		1.0471	nr	1.4049	nr	1.1899
Joliette PQ	river	30Oct-2Nov	1	24	25	1.2094	nr	nr	1.0805	1.1823	1.2195	1.1922
Wayagamack PQ	river	1-7 Oct	2	8	9	1.3204	1.1249	1.0526	nr	1.2228	1.1778	1.2000
Bromptonville PQ	river	nr	3,2	17	25	nr	nr	nr	1.0682	1.2207	1.2647	1.2057
Edmunston NB	river	19-29 Sept	1,3	63	302	nr	nr	1.0386	1.0903	1.1400	1.6450	1.2244
Pontiac PQ	river	23-29 Sep	2	13	4	nr	1.1589	1.0628	1.0755	1.2318	1.2023	1.2318
Pine Falls MB	river	25Aug-5Sep	1	23	35	1.3961	1.2471	1.0517	1.1482	nr	1.2271	1.2589
Windsor PQ	river	7-14 Sept	3,2	40	37	nr	nr	nr	1.1490	1.2782	1.3618	1.2874
Dryden ON	river	20-27 Oct	2	20	20	1.2543	1.3041	1.0819	1.0837	1.2572	1.2318	1.2921
Kingsey Falls PQ		nr	nr	16	17	1.2617	nr	nr	1.0763	1.2813	1.3111	1.3387
Nackawic NB	river	Oct	1	25	25	1.4101	1.2247	1.0619	1.0778	1.2293	nr	1.4263
Lennoxville PQ	river	22-27 Sep	3	23	22	2.9011	1.4254	1.1497	1.1821	1.4248	2.4541	1.5248
Crabtree PQ	river	11-21 Sep	3	24	20	2.2842	8.7534	nr	1.1947	1.5892	nr	1.8292
La Tuque PQ	river	7-29 Oct	2	21	19	2.5186	1.4429	1.1146	1.3009	1.7505	2.5582	2.2566
Portneuf PQ	river	Sep-Oct	3	25	23	2.5149	1.9681	1.2405	1.1444	1.4763	1.4763	2.6556
PontRouge PQ	river	18-23 Oct	1	20	10	1.3712	1.5619	1.1525	1.2661	1.5949	5.1970	3.8011
Thunder Bay ON	river	19-26 Sep	2	20	20	1.4299	1.1772	1.0528	1.0723	1.2892	1.2062	nr
Thunder Bay ON	lake	10-24 Sep	2	32	20	nr	nr	1.0517	1.0936	1.3205	1.2258	nr
Gatineau PQ	river	25Oct-4Nov	2	17	22	1.8310	1.2441	1.2287	1.1085	1.1768	1.4071	nr
St-Raymond PQ	river	21-31 Aug	2,5	24	3	nr	nr	nr	nr	nr	nr	nr
Lebel PQ	river	16-22 Sep	5	4	12	nr	nr		nr	nr	nr	nr
Clermont PQ	river	Aug, Oct	2	40	10	1.4811	2.2989	1.3478	1.4881	2.4489	nr	
Prince Albert SK	river		3	21	20	1.6561	SI	nr	1.1768	1.4801	1.7494	2.1833
Red Rock ON	lake	26Sep-2Oct	1	43	51	1.3573	nr	1.0347	1.0682	1.2064	1.1778	1.1879
Terrace Bay ON	river	22-28 Sept	2	24	20	1.3689	1.2846	1.0794	1.0827	1.1936	1.3133	1.3668

Minimum	1.2094	1.1249	1.0347	1.0682	1.1400	1.1778	1.1812
Maximum	2.9011	8.7534	1.3478	1.4881	2.4489	5.1970	3.8011
Median	1.4031	1.2944	1.0623	1.0936	1.2782	1.2879	1.2898

B. Male white sucker.

Mill	Receiving	Dates	Method	Number Reference	Number Exposed	antilog RMSE age	antilog RMSE BW	antilog RMSE L	antilog RMSE Cond	antilog RMSE LW	antilog RMSE GW
Joliette PQ	river	30Oct-2Nov	1	25	24	1.2426	nr	nr	1.0655	1.2456	1.1686
Dryden ON	river	20-27 Oct	2	5	20	1.3772	1.3608	1.0669	1.0767	1.2971	1.1936
Gatineau PQ	river	25Oct-24Nov	2	11	20	1.2869	1.2125	1.1448	1.0755	1.1953	1.1953
Lebel PQ	river	16-22 Sep	5	3	18	1.4578	1.1140	1.0471	1.0715	1.2023	1.2040
Laurentide PQ	river	30Sep-7Oct	2	6	3	nr	2.0855	1.0528	2.0516	1.5904	1.2174
Pine Falls MB	river	25Aug-5Sep	1	25	36	1.4496	1.2174	1.0565	1.1168	nr	1.2732
Thunder Bay ON	river	20-27 Oct	2	20	20	1.3524	1.1674	1.0575	1.0655	1.2817	1.3306
Bromptonville PQ	river	nr	3,2	13	14	nr	nr	nr	1.0427	1.2928	1.4378
Kingsey Falls PQ		nr	nr	7	23	1.3791	nr	nr	1.0651	1.2339	1.5094
Lennoxville PQ	river	22-27 Sep	3	21	23	2.0917	1.5940	1.1462	1.1714	10.5112	1.5445
Kenora ON	river	22-29 Sep	2	14	25	1.4282	1.1774	1.0560	1.0993	1.2237	1.6084
Portneuf PQ	river	Sep-Oct	3	23	22	2.6746	1.9168	1.2391	1.1173	1.4072	1.6285
Pontiac PQ	river	23-29 Sep	2	4	7	nr	1.2441	1.0528	1.0755	1.3093	1.6395
Edmunston NB	river	19-29 Sept	1,3	35	231	nr	nr	1.0371	1.0690	1.1405	1.6421
Crabtree PQ	river	11-21 Sep	3	20	16	2.8314	2.1221	1.2634	1.2274	1.7878	1.7065
Thunder Bay ON	lake	10-24 Sep	2	8	20	nr	nr	1.0585	1.1138	1.2411	1.8467
St-Raymond PQ	river	21-31 Aug	2,5	13	27	1.3678	nr	nr	1.1220	nr	1.9343
Trois-Rivieres PQ	river	1-7 Oct	2	5	7	1.6702	1.4054	1.1104	1.1088	1.2417	2.0492
Nackawic NB	river	Oct	1	25	25	1.4246	1.2354	1.0655	1.0747	1.2711	2.5356
Windsor PQ	river	7-14 Sept	3,2	40	34	nr	nr	nr	1.1113	1.2684	nr
La Tuque PQ	river	7-29 Oct	2	18	20	2.3421	1.6296	1.1940	1.2498	1.4446	nr
Prince Albert SK	river			23	21	1.4286	1.0447	nr	1.2125	1.5665	2.2055
Terrace Bay ON	river	22-28 Sept	2	25	13	1.4001	nr	nr	nr	1.2605	2.0846
Red Rock ON	lake	26Sep-2Oct	1	49	29	1.2588	nr	1.0276	1.0580	1.1866	1.1391
Minimum						1.2426	1.0447	1.0276	1.0427	1.1405	1.1391
Maximum						2.8314	2.1221	1.2634	2.0516	10.5112	2.5356
Median						1.4264	1.2441	1.0585	1.0993	1.2697	1.5765

C. Female longnose sucker.

Mill	Receiving	Dates	Method	Number Reference	Number Exposed	antilog RMSE age	antilog RMSE BW	antilog RMSE L	antilog RMSE Cond	antilog RMSE LW	antilog RMSE GW	antilog RMSE Fecund
Beaupre PQ	river	Aug	2,3	20	20	1.3166	1.1521	1.0414	1.0923	1.2799	1.3608	nr
Lebel PQ	river	16-22 Sep	5	14	14	9.0000	nr	nr	1.0580	nr	1.3596	nr
Amos PQ	river			20	20	1.8189	1.4771	1.1414	1.1650	1.4619	1.6605	nr
Portneuf PQ	river	Sep-Oct	3	22	21	1.9926	1.8055	1.2133	1.1706	1.3961	1.4881	nr
St-Raymond PQ	river	21-31 Aug	2,5	13	33	1.2703	nr	nr	1.1806	nr	1.5610	1.3950
Laurentide	river	30Sep-7Oct	2	5	8	nr	1.2869	1.0830	1.1056	1.2380	2.8884	1.2604
Hinton AB	river			45	15	1.2444	1.1819	1.0575	1.0819	1.2074	1.1664	1.3382
Grande Prairie SK	river	23Aug-Sep	3	45	22	1.2529	1.0528	nr	1.0876	nr	1.1686	1.2973
Taylor BC	river	Aug	3	20								
Jonquieres PQ	river	18-26 Sep	1	11	99	1.7185	1.7486	1.1862	1.4360	2.1312	nr	nr
Donnacona PQ	lake	11-16 Oct	1	20	20	1.5366	1.3208	1.0794	1.2318	1.7848	1.5730	1.6578
Red Rock ON	lake	26Sep-2Oct	1	43	20	1.3543	1.0355	nr	1.0727	1.1891	1.2104	1.1825
Terrace Bay ON	river	22-28 Sep	2	15	23	1.3987	1.2951	1.0886	1.0786	1.2377	1.6360	nr
Minimum						1.2444	1.0355	1.0414	1.0580	1.1891	1.1664	1.1825
Maximum						9.0000	1.8055	1.2133	1.4360	2.1312	2.8884	1.6578
Median						1.3987	1.2910	1.0858	1.0989	1.2799	1.4881	1.3178

D. Male longnose sucker.

Mill	Receiving	Dates	Method	Number Reference	Number Exposed	antilog RMSE age	antilog RMSE BW	antilog RMSE L	antilog RMSE Cond	antilog RMSE LW	antilog RMSE GW
Beaupre PQ	river	Aug	2,3	20	20	1.2992	1.1945	1.0605	1.0900	1.3110	1.4079
Lebel PQ	river	16-22 Sep	5	11	7	nr	nr	nr	1.0933	nr	nr
Amos PQ	river			21	20	1.8803	1.3620	1.0996	1.1482	1.4475	nr
Taylor BC	river	Aug	3	>20					1.0000	1.0000	1.0000
Portneuf PQ	river	Sep-Oct	3	22	22	nr	1.3787	1.1215	1.1079	1.3438	1.4248
Laurentide PQ	river	30Sep-7Oct	2	3	5	nr	1.2191	1.0866	1.0996	1.4423	1.2207
Hinton AB	river			28	7	1.2391	1.1205	1.0363	1.0731	nr	1.5457
Grande Prairie SK	river	23Aug-10Sep	3	30	39	1.2295	1.0420	nr	1.1090	1.2612	1.5056
Donnacona PQ	lake	11-16 Oct	1	20	20	1.6794	1.3770	1.0830	1.2486	2.8667	nr
Terrace Bay ON	river	22-28 Sep	2	13	17	1.3303	1.3009	1.0841	1.0624	1.2181	1.6539
Minimum						1.2295	1.0420	1.0363	1.0000	1.0000	1.0000
Maximum						1.8803	1.3787	1.1215	1.2486	2.8667	1.6539
Median						1.3147	1.2600	1.0841	1.0964	1.3274	1.4248

E. Female walleye.

Mill	Receiving	Dates	Method	Number Reference	Number Exposed	antilog RMSE age	antilog RMSE BW	antilog RMSE L	antilog RMSE Cond	antilog RMSE LW	antilog RMSE GW	antilog RMSE Fecund
Dryden ON	river	20-27 Oct	2	17	20	1.2543	1.3041	1.0819	1.0837	1.2572	1.2318	1.2921
Iroquois Falls ON	river	19-24 Sep	2	20	20	nr	nr	1.0946	1.0869	1.2979	1.8159	nr
Lesser Slave AB	river	Sep/89-94	3									
Prince Albert SK	river		3	21	2	1.9953	1.5384	nr	1.1568	1.2869	nr	nr
Temiscaming PQ	river	21-31 Aug	2	20	10	2.3267	3.0366	1.3666	1.2174	1.5337	nr	nr
Minimum						1.2543	1.3041	1.0819	1.0837	1.2572	1.2318	1.2921
Maximum						2.3267	3.0366	1.3666	1.2174	1.5337	1.8159	1.2921
Median						1.9953	1.5384	1.0946	1.1218	1.2924	1.5239	1.2921

F. Male walleye.

Mill	Receiving	Dates	Method	Number Reference	Number Exposed	antilog RMSE age	antilog RMSE BW	antilog RMSE L	antilog RMSE Cond	antilog RMSE LW	antilog RMSE GW
The Pas MB	river	26Sep-3Oct	1	23	33	1.6075	1.3264	1.1038	1.1620	nr	1.6204
Dryden ON	river	20-27 Oct	2	16	21	1.3772	1.2840	1.0669	1.0767	1.2971	1.1936
Iroquois Falls ON	river	19-24 Sep	2	20	20	nr	nr	1.0873	1.0873	1.2176	1.6529
Thunder Bay ON	river	20-30 Oct	2	20	22	1.3695	nr	nr	1.0575	1.3433	1.5175
Lesser Slave AB	river	Sep/89-94	3								
Temiscaming PQ	river	21-31 Aug	2	20	30	2.1185	1.9289	1.2141	1.1806	1.6298	nr
Prince Albert SK	river		3	20	10	2.3595	1.6648	nr	1.1568	1.5194	SI
Minimum						1.3695	1.2840	1.0669	1.0575	1.2176	1.1936
Maximum						2.3595	1.9289	1.2141	1.1806	1.6298	1.6529
Median						1.6075	1.4956	1.0955	1.1220	1.3433	1.5690

G. Female winter flounder.

Mill	Receiving	Dates	Method	Number Reference	Number Exposed	antilog RMSE age	antilog RMSE BW	antilog RMSE L	antilog RMSE Cond	antilog RMSE LW	antilog RMSE GW	antilog RMSE Fecund
Stephenville NF	estuary	29Jul-4Aug	2	20	19	nr	nr	1.1601	1.1118	1.2334	1.2787	nr
East River NS	estuary	6-15 Aug	2	25	25	1.2973	1.4358	1.1215	1.0999	1.2377	1.2925	nr
Dalhousie NB	esturary	17-26 Aug	2	26	21	1.3194	1.6450	1.1464	nr	2.2549	1.4301	nr
Pictou NS	coast	26-Sep	8	31	36	nr	nr	1.5572	2.1907	3.0968	3.8518	nr
Corner Brook NF	estuary	13-15 Jul	2	30	47	1.3432	1.4063	1.1044	1.1085	1.2069	1.2605	nr

Pt. Hawkesbury NS	coast	20-24 Jun	2	20	21	1.2963	nr	1.1484	1.0971	1.2133	1.9124	nr
New Richmond PQ		15-21 Sep	1	30	18	nr	1.3781	1.0794	1.1609	1.4558	SI	nr
Bathurst NB	esturary	19-22 Sep	gillnets	19		1.6734	1.3849	1.0000	1.0000	1.3849	1.9180	nr
Minimum						1.2963	1.3781	1.0000	1.0000	1.2069	1.2605	nr
Maximum						1.6734	1.6450	1.5572	2.1907	3.0968	3.8518	nr
Median						1.3194	1.4063	1.1339	1.1085	1.3113	1.4301	nr

Note: ABT and Avenor fish had undeveloped gonads

H. Male winter flounder.

Mill	Receiving	Dates	Method	Number Reference	Number Exposed	antilog RMSE age	antilog RMSE BW	antilog RMSE L	antilog RMSE Cond	antilog RMSE LW	antilog RMSE GW
Stephenville NF	estuary	29Jul-4Aug	2	20	21	nr	nr	1.1236	1.0974	1.1882	1.2651
East River NS	estuary	6-15 Aug	2	24	25	1.3186	nr	nr	nr	1.6129	1.4787
Dalhousie NB	esturary	17-26 Aug	2	13	12	1.1806	1.7594	1.1791	1.0869	1.2804	1.6781
Pictou NS	coast	26-Sep	8	9	4	nr	nr	1.3961	1.7742	2.1312	2.9953
Corner Brook NF	estuary	13-15 Jul	2	26	36	1.4087	1.3448	1.0855	1.1332	1.1716	1.1893
Pt. Hawkesbury NS	coast	20-24 Jun	2	12	20	1.2385	nr	1.1191	1.1082	1.1995	1.3324
New Richmond PQ		15-21 Sep	1	10	12	nr	1.1670	1.0471	SI	1.4233	2.4844
Minimum						1.1806	1.1670	1.0471	1.0869	1.1716	1.1893
Maximum						1.4087	1.7594	1.3961	1.7742	2.1312	2.9953
Median						1.2786	1.3448	1.1214	1.1082	1.2804	1.4787

Note: ABT and Avenor fish had undeveloped gonads

I. Female tomcod.

Mill	Receiving	Dates	Method	Number Reference	Number Exposed	antilog RMSE age	antilog RMSE BW	antilog RMSE L	antilog RMSE Cond	antilog RMSE LW	antilog RMSE GW	antilog RMSE Fecund
Hantsport NS	estuary	6-Jul	8	20	22	1.2963	1.3849	1.1568	1.1568	1.4901	1.3849	nr
Miramichi NB	estuary	20-21 Oct	2	19	28	nr	1.3381	nr	1.0755	1.2125	1.1953	nr
Miramichi NB	estuary	20-21 Oct	2	25	28	nr	1.4999	nr	1.1085	1.2287	1.2869	nr
Miramichi NB	estuary	29Oct-1Nov	2	19	20	nr	1.4444	nr	1.0965	1.2091	1.2632	1.5702
Miramichi NB	estuary	29Oct-1Nov	2	19	20	nr	nr	nr	nr	1.2287	nr	1.6289
Bathurst NB	estuary	19-22 Sep	gill nets	15		nr	1.2589	0.0000	1.0000	1.4901	1.8390	nr
Minimum						1.2963	1.2589	0.0000	1.0000	1.2091	1.1953	1.5702
Maximum						1.2963	1.4999	1.1568	1.1568	1.4901	1.8390	1.6289

Median	na	1.3849	0.5784	1.0965	1.2287	1.2869	1.5996

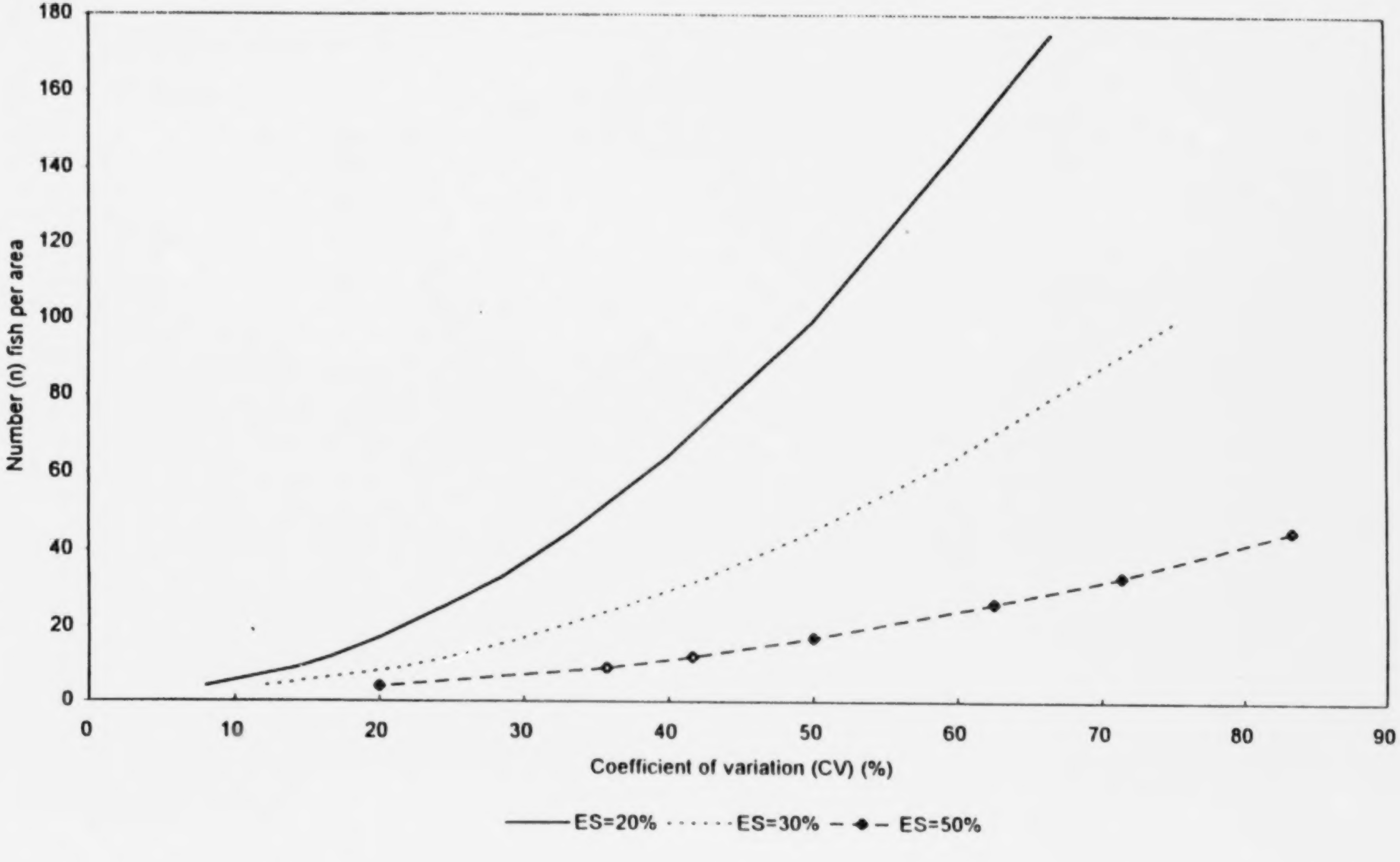
Note: Minas Basin and Stone Bathurst fish had undeveloped gonads.

J. Male tomcod.

Mill	Receiving	Dates	Method	Number Reference	Number Exposed	antilog RMSE age	antilog RMSE BW	antilog RMSE L	antilog RMSE Cond	antilog RMSE LW	antilog RMSE GW
Hantsport NS	estuary	6-Jul	8	20	22	1.2647	1.2589	1.1568	1.1568	1.5849	1.8390
Miramichi NB	estuary	20-21 Oct	2	20	20	nr	1.2732	nr	1.0755	1.5289	1.1568
Miramichi NB	estuary	20-21 Oct	2	22	20	nr	1.3502	nr	1.0755	1.5384	1.1768
Bathurst NB	estuary	19-22 Sep	gill nets	18		nr	2.0712	1.0000	1.0000	2.0712	1.3849
Miramichi NB	estuary	29Oct-1Nov	2	21	8	nr	nr	nr	nr	1.4731	1.1568
Miramichi NB	estuary	29Oct-1Nov	2	10	8	nr	1.3513	nr	0.0009	1.5318	nr
Minimum						1.2647	1.2589	1.0000	0.0009	1.4731	1.1568
Maximum						1.2647	2.0712	1.1568	1.1568	2.0712	1.8390
Median						1.2647	1.3502	1.0784	1.0755	1.5351	1.1768

Note: Minas Basin and Stone Bathurst fish had undeveloped gonads.

Figure 1
n vs CV for ES=20,30,50% (power=0.8)



when it cannot be assumed that the true population variances will not also change over time. However, if sample sizes for any ES are calculated for the two species, *n* for CV=30% will be approximately double those for CV=20%. Similarly, sample sizes required for detecting an ES of 20% would be approximately double those for detecting an ES of 30% (Figure 1), yet there is arguably little difference in ecological significance between the two ES.

It is much better to calculate CV, ES or *P* for some reasonable range of *n* such as 10-30 for the Fish Survey in a qualitative sensitivity analysis. This type of approach usually reveals that there is little to be gained by increasing sample sizes beyond 20, and that the most cost-effective way to improve power in most studies is to reduce variances. After the study has been conducted, the confidence limits for the difference between reference and exposed areas should be calculated and reported even if the difference is not statistically significant (Paine et al., 1996).

Finally, what is the power of field surveys, and specifically this Fish Survey, relative to laboratory toxicity tests? Table 14 provides average effect size, as % reductions, at MATC (Maximum Acceptable Toxic Concentrations) from a review of laboratory fish toxicity tests (Suter et al., 1987). The effect size given are roughly equivalent to detectable effect size for P=0.5, using a one-tail test with α=0.05 (Appendix II). There is a common misperception that laboratory tests are more powerful than field surveys. This is simply not true for measurements of effects on reproduction and growth (weight in Table 14 is assumed to represent growth). Most Cycle One Fish Surveys with $n\geq20$, and some with smaller *n*, would have more power than the "mean" toxicity test to detect reproductive and growth effects. However, the laboratory tests would probably have more power to detect effects on adult and larval survival. Finally, the effect size at the MATC in Table 14, and consequently the power of the laboratory tests, varied widely among studies within variables. Power would also vary widely among studies in the Fish Survey, based on Cycle One results (e.g., Table 13), so the most appropriate conclusion would be that the largest differences in power exist among laboratory tests or among field surveys rather than between the laboratory and the field.

Other Statistical Issues for Cycle Two and Multi-Cycle Fish Survey

In a multi-cycle EEM program, industry, regulators and consultants must also consider higher-order variances. If FS (or any other studies) are conducted over several cycles, the issue of whether differences between reference and exposed areas, or more generally, ES, are stable over time must be addressed (=spatial-temporal interactions). In a multi-cycle FS, it is not unreasonable to expect some temporal variation of any natural differences between areas. A real effect from a mill discharge might also vary because, e.g., effluent dilution varies among cycles.

Table 14. Power of fish laboratory toxicity tests (from Suter *et al*, 1987).

Endpoint	No. tests	% reduction at MATC		
		Mean	Min.	Max.
Parental survival	13	20	<=0.1	61
Hatch	16	12	<=0.1	69
Fecundity	27	42	<=0.1	87
Larval survival	40	19	<=0.1	71
Weight (growth)	52	20	<=0.1	44
Egg weight	24	35	3	71
Lowest observed effect	77	28	<=0.1	84

NOTE: MATC = Maximum Acceptable Toxicant Concentration

There are some potential statistical problems associated with repeating the FS over several cycles, discussed in more detail in Appendix II. If sample sizes within cycles are increased or variances within areas are reduced (both commendable), the power to detect a difference between areas increases within any cycle. However, over several cycles, the power to detect natural or impact-related spatial-temporal interactions also increases. Consultants analyzing multi-cycle FS data should be aware of this paradox, and avoid over-interpretation of statistically significant but small interactions (and differences between areas). There are also some minor issues concerned with the appropriate error term for tests of effects (i.e., differences between areas). However, these and other issues for multi-cycle FS are trivial compared to those for multi-cycle Invertebrate Community Survey (ICS). Basically, there will be large and statistically significant spatial-temporal interactions, natural and impact-related, in almost any ICS (see, e.g., many papers in Vol. 18 {1993} and Vol. 20 {1995} of the Australian Journal of Ecology).

4.0 SECOND CYCLE CONSIDERATIONS

4.1 Second Cycle Objectives

The first objective is to obtain sufficient numbers of resident fish from reference and exposed areas with similar habitats. Once that has been achieved, the next objective is to identify the presence of site-related changes in age structure, growth and reproduction in fish as measured by life history variables. It must be recognized that any interpretation will depend on a weight of evidence accumulated with multi-cycle data. For a survey to accomplish this objective, fish must be present, they must be exposed for a significant portion of their life cycle and the receiving environment must allow a good level of interpretation of the data. If these conditions cannot be met then alternatives given in Sec. 4.3 should be considered.

It must be recognized that it is not cost-effective to collect information at multiple levels of organization on the fish in the receiving environment, so some sort of conservative program must be developed. Based on previous studies of fish in receiving waters of pulp mills the dominant concern with respect to impacts on fish populations is detection of changes in fish reproductive performance. Previous studies of fish in pulp mill discharge environments have observed 25-50% differences in relative gonad weights, compared to reference fish. The FS EWG felt that 25% was a conservative indicator of change, but that choosing a single target number could be misleading. Since variability would differ between cycles, the EWG also felt that it was important that success of collections not be determined solely by the ability to meet the target of 25%. It was decided that a range of 20-30% would be a target range for gonad size differences.

Since different parameters have different levels of variability, it is not possible to design a program which would require different levels of effort for different measurements. A conservative power level would be 0.8. The magnitude of the difference which could be detected for other parameters would be fixed based on the sample size required for determining an effect on gonad size. The power for detecting differences in other parameters should be reviewed during study design to ensure that reasonable power is achieved for as many variables as possible. Studies must be designed site-specifically and the EWG recommends that priority be given to reducing variability rather than increasing sample sizes beyond Cycle 1 requirements in order to satisfy the objective of this survey.

Target effect sizes for growth rather than reproduction are needed for those cases where juvenile fish are surveyed.

It would be of benefit to the EWG to have an overview of requirements from the other EWGs in formulating their final requirements. The second and subsequent cycles of EEM should provide data of sufficient quality to build a database which will be used to examine the effectiveness of the regulations after a number of cycles.

4.2 Decision Process for Study Design Cycle Two (Decision Tree)

Recommended Changes for Annex 1 document. THESE WERE THE RECOMMENDATIONS OF THE EWG FOR THE TMC. THE ANNEX TO THE REGULATION SHOULD BE CONSULTED FOR DETERMINATION OF THE FINAL REQUIREMENTS.

Annex 1.0 Requirements

EFFECTS ON FISH: METHOD AND REPORTING REQUIREMENTS FOR THE ADULT FISH SURVEY

The fish survey provides an initial assessment of whether there are differences in fish characteristics between exposed and reference sites. It does not attempt to provide a comprehensive assessment of the health of a fish population. Since reproductive impacts are the primary concern, the study design should be based on the ability to detect a difference of 20-30% in relative gonad size between sites. Priority should be given to reducing variability rather than increasing sample size. A reasonable level of fishing effort should be expended to collect the minimum number of individuals specified. Except in cases of extreme habitat degradation, it is unlikely that one survey alone would provide sufficient data on which to judge the effects of a mill effluent on the receiving environment. Rather, it will be the results of a series of such surveys, conducted every three years, that will permit such an assessment.

The criteria for sentinel species are that they are abundant, exposed and can provide measurements for the desired characteristics (Table 15). For the first undertaking of EEM, only a near-field area (*i.e.*, within the predicted zone of any sublethal effects or in the immediate vicinity of the effluent discharge) and a reference area were sampled. In subsequent EEM cycles, fish survey designs will be improved based on previous results. The presence or absence of differences can only be determined in most cases given the presence of two cycles of data with sufficient power. The results of Cycle One should be used to site-specifically design the Cycle Two requirements (Decision tree, Fig. 2) based on Tables 16-21.

The fish survey need only be undertaken once during the first EEM cycle. Additional sampling attempts may be necessary if the first collection is unsuccessful. Timing of collections should be such that fish in the near-field area are likely to have had prolonged exposure to the effluent. Consequently, sampling should be avoided during the spawning period, including immediate pre-spawning and post-spawning periods.

Table 15. ADULT FISH SURVEY MEASUREMENTS

Measurement Requirement	Expected Precision	Reporting Requirement
Length (standard, total and fork)	+/- 0.2cm	Individual measurements, mean, standard deviation
Fresh weight	+/- 5.0%	Individual measurements, mean, standard deviation
Age	+/- 1 year (10% to be independently confirmed)	Individual measurements, mean, standard deviation
Gonad weight	+/- 1.0%	Individual measurements, mean, standard deviation
Egg size	+/- 1.0%	Weight, minimum subsample sizes of 100 eggs
Weight of liver or hepatopancreas	+/- 1.0%	Individual measurements, mean, standard deviation
External condition	NA	Obvious abnormalities, prevalence of lesions, tumours, parasites, etc.

Fish Survey Decision Tree

The Fish Survey decision tree (Fig. 2) has been developed by the FS EWG to aid government, industry and consultants in deciding on the most appropriate Cycle Two design for specific sites. The tables referred to in the tree (Tables 16-21) provide further details and guidance for decision-making and are presented following the tree.

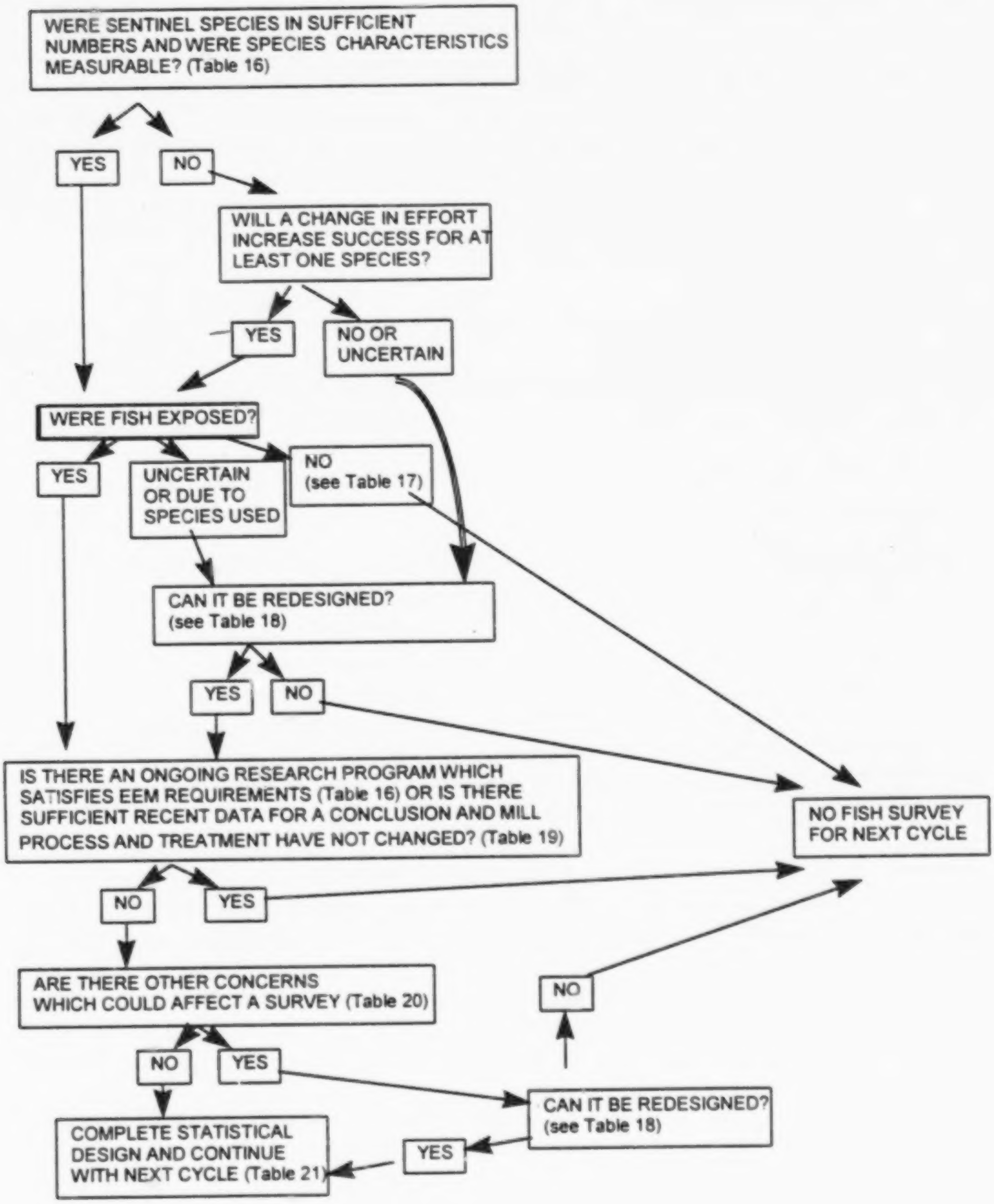

WERE SENTINEL SPECIES IN SUFFICIENT NUMBERS AND WERE SPECIES CHARACTERISTICS MEASURABLE? (Table 16)
YES
NO
WILL A CHANGE IN EFFORT INCREASE SUCCESS FOR AT LEAST ONE SPECIES?
YES
NO OR UNCERTAIN
WERE FISH EXPOSED?
YES
UNCERTAIN OR DUE TO SPECIES USED
NO (see Table 17)
CAN IT BE REDESIGNED? (see Table 18)
YES
NO
IS THERE AN ONGOING RESEARCH PROGRAM WHICH SATISFIES EEM REQUIREMENTS (Table 16) OR IS THERE SUFFICIENT RECENT DATA FOR A CONCLUSION AND MILL PROCESS AND TREATMENT HAVE NOT CHANGED? (Table 19)
NO FISH SURVEY FOR NEXT CYCLE
NO
YES
ARE THERE OTHER CONCERNS WHICH COULD AFFECT A SURVEY (Table 20)
NO
NO
YES
CAN IT BE REDESIGNED? (see Table 18)
COMPLETE STATISTICAL DESIGN AND CONTINUE WITH NEXT CYCLE (Table 21)
YES

Figure 2. Decision flow chart for the fish survey component for Cycle Two.

Table 16. Sentinel species characteristics and criteria for success

Criteria for defining "sufficient numbers" and "suitable characteristics" of sentinel fish species

a) were 20 adults of each sex of at least one species caught in each area? If yes, go to b)

b) was it possible to measure all fish survey parameters on the fish?
  - age
  - body weight and length
  - liver or hepatopancreas weight
  - gonad weight
  - fecundity and ovum size(?)

c) were gonad weights of at least 80% of the female fish greater than 1% of the carcass weight?

d) if the sentinel species is a fractional spawner are you confident that sampling was not during the spawning period?

Bivalves: some species will not meet these criteria because gonads are difficult to measure

Table 17. Criteria for defining high dilution

If fish were captured in the effluent field, then exposure is obvious, although the period of residency is unknown. In situations where large, statistically significant differences are seen between exposure and reference sites, it is obvious that there has been a significant period of residency and and an absence of mixing of fish between sites. Although these differences may not be the result of exposure to pulp mill effluents, they do indicate some significant period of residency in the effluent field, and an absence of mixing between reference and exposure fish. The main concern relates to establishing the period of residency in the area of the effluent field when no obvious barriers are present and there are no differences between sites. Most chemical and biochemical tracers only provide evidence of short-term residency (days to weeks) and they were not used very successfully in the first cycle. The real value of chemical tracers is to establish that reference fish did not spend significant time in the effluent field. If there is doubt about the exposure, then redesign of the survey should be considered to utilize fish which are more likely to remain longer in the effluent field.

Surveys should not be conducted in the case of high dilution:

i) In rivers, is effluent concentration, based on relative flows, <0.1% after complete mixing at low flow, and is the effluent diluted to <1% within 100-250 m of the discharge?

ii) in marine and lake systems, is the effluent diluted to <1% within 100-250 m of the discharge?

Table 18. Guidance for re-design and alternatives

1. If fish exposure was uncertain
   a) are less mobile fish (including small-bodied species) available, which would increase likelihood of exposure? If no, go to b)
   b) if no other species are available would changing the time of sampling increase the likelihood of exposure, using the same species as in Cycle One? If no, go to 2.

2. Adult fish are a problem. If other approaches approaches are not appropriate, then go to 3.

3. Survey can not be redesigned.

Table 19. Criteria for sufficient and recent data for a conclusion.

The purpose of the EEM program is to evaluate the adequacy of the regulation. There are three possible conclusions which can be reached: there is no effect, there is a mill-related effect, or there are confounding factors which prevent determination of the cause of an effect.

1. For determining that there are or are not changes, a minimum of 2 cycles, with a power and effect size that satisfy the requirements of Table 21, are required. Supplementary data could be used to replace one cycle of EEM data if it provides the required information on age, growth and reproduction, and has been collected since the last major process change.

2. Confounding factors - if there is sufficient data to demonstrate that other discharges or contaminant sources are primarily responsible for observed changes or absence of observed changes, and the survey cannot be re-designed to isolate pulp mill impact, then there can be a conclusion that confounding factors prevent interpretation for the purposes of assessing the regulation.

3. Input is required by the CPPA to define sufficient process changes which would warrant further sampling.

4. A change in the receiving environment (for example, reservoir impoundment) may trigger initiation of a subsequent cycle.

Table 20. Other concerns which could affect a survey.

Other dischargers are present and survey cannot be re-designed to isolate impact. Some examples include:

i) mill effluent is a minor component (*e.g.* less than 20%) of a combined discharge

ii) the mill discharge is located within the effluent field of an adjacent discharger or and/or adjacent discharger is located within the mill effluent field, and the mill discharge represents a minor component (*e.g.* less than 20%) of the discharges into the effluent field, and the survey cannot be re-designed to isolate the mill impact

iii) other confounding factors may exist which have not been identified and it was not the intention of the EWG to exclude factors which caused substantial confounding.

Habitat unsuitable or unsafe for sampling:
e.g. extreme tidal fluctuations, high current velocity, etc.

No suitable reference site
e.g. due to habitat, fish movements (reference fish exposed)

Permit restrictions or concerns about protected, rare, or endangered species

Restrictions on sampling such as timing or location preventing sampling of appropriate species or correct timing

Table 21. Power analyses

The sample sizes should be determined using the variability estimates given in Cycle One, with a target effect size of 0.8. If variability estimates are not available, a sample size of 30 males and 30 females from each site would be conservative.

Table 22. Possible alternatives

a) If no adult fish are resident and exposed, are juvenile fish available and can 60 be collected for determining relative growth rates, abundance and deformities? If no, go to b)
b) Is the area a known spawning area, and can a protocol be provided for a survey of larval fish abundance, relative to reference areas, be conducted and provide defensible data? If no, go to c)
c) Are bivalves resident in the area, and can a protocol be defined that provides defensible endpoints for a wild bivalve survey? If no, go to d)
d) Are there other alternatives (e.g. caged bivalves, mesocosm exposure) which are not research and can provide suitable data? If no, go to 3.

4.3 Further Research Needs

There are a number of data gaps that need to be filled to expand the capability of the Fish Survey. These have been divided into data gaps related to a) capture, b) exposure and c) interpretation.

a) Fish Capture

i) mobility and migration/ migratory species
Fish mobility in open systems was identified as an issue at a large number of sites, and tracers were unsuccessful at demonstrating exposure. The issues related to mobility can be divided into issues related to the mobility of widely used species, mobility of

marine species, and the mobility of small-bodied fish species. Research programs are needed to address these issues.

ii) defining effort

Fishing effort problems were associated with not capturing sufficient numbers of fish, and with associated with capturing fish with high amounts of variability.

- *defining fishing techniques*
  The method for calculating fishing effort varied dramatically between surveys, but the EWG was not comfortable giving guidance on how to calculate fishing effort, given the variability in methods, receiving environments, and types of gear. It was obvious that some unsuccessful surveys were related to inadequate fishing effort and/or gear.
- *defining variability* (natural versus sampling-induced or sampling-reduced)
  Reducing variability of the FS parameters is probably the most effective means of reducing costs or increasing power in Cycle Two. Some potentially controllable factors which led to increased variability were identified and discussed by the EWG, but these factors probably need to be evaluated site-specifically. Further review of Cycle One data, and the literature, on factors affecting variability should be conducted, and guidance provided for Cycle Two.

a) Fish Exposure

i) exposure or lack of exposure - tracers

Research needs related to confirming exposure of fish to mill effluents have been addressed by the Tracer EWG.

b) Interpretation

i) timing of sampling:

Little is known about using marine fish in Canadian receiving waters for monitoring purposes. It was evident during the first cycle that most marine fish species used show little gonadal development six months prior to spawning, and put their energy into gonadal growth quite late in development. Research is needed on improving the timing of sampling. Sampling at different times of the year will change the variability and there has to be a trade-off between sampling time, variability, stages of reproductive development and mobility. Research is needed on how to optimize sampling to achieve maximal relevance, residency period and minimal variability and sampling costs.

ii) range in natural variability versus potential responses to effluent exposure:

There is concern about the natural ranges within and between populations in the parameters being measured. Research is required on multiple reference sites, and the ranges in variability which are seen, relative to the differences seen between exposed and reference sites.

iii) multiple discharge environments:

Confounding discharges were common in the first cycle, and research is needed on how to discriminate between impacts from different effluents when they are discharged in close proximity.

iv) timing or frequency of sampling for forage species which are multiple spawners:

It may be difficult to identify the total reproductive potential of multiple-spawning species from a single sampling period. Estimates from a single sample are assumed to be a conservative estimator, but may miss some subtle impacts which would affect the clutch size of subsequent efforts. Research is required to identify the optimal manner of estimating reproductive potential in fractional or multiple spawners, and to determine whether multiple sampling trips would be required for these small-bodied species to get a more accurate assessment.

v) link between physiological/morphological changes and responses at the population or community level:
It is a research need outside of this program to develop the information which could tie together suborganismal, organismal, population and community-level responses. A joint government-industry research program is required to evaluate the relative strengths, weaknesses and effectiveness of the various approaches.

vi) interpretation of some proposed alternatives:
mesocosm exposures: Pilot scale studies are needed to evaluate the ability of this tool to be used cost-effectively as a replacement for a FS when the complexity of the receiving environment prevents adequate interpretation. Laboratory assays have successfully demonstrated that chronic exposures will predict the reproductive responses of concern. While mesocosms have been successfully used to measure the impact of pulp mill effluent on algal and benthic invertebrate communities in freshwater rivers in Prince George, BC and Hinton, Alta. (Culp et. al. 1996; Culp and Podemski 1995), adaptation of the system to the estuarine environment and fish remains to be accomplished. A proposal to carry out this work in New Brunswick is described in Dubé and Culp (Appendix III) and is scheduled to be carried out in 1997.
aging of crabs/oysters/lobsters: The inability to age crustaceans handicaps their utility for information on maturity and growth rates. Research is needed to review the literature to determine if there are acceptable methods for obtaining this information in a cost-effective manner which would be applicable to the FS component of an EEM cycle.
scope for growth issues: While scope for growth provides valuable information the EWG feels that more research is needed to implement this as an EEM tool.
caged bivalves: Transplanted mussels and barnacles have been used as a monitoring tool at a coastal marine pulp mill in Canada (Wu and Levings, 1980). Two pilot projects testing the application of caged bivalves for monitoring pulp mill effluents in Atlantic estuaries are proposed. The first, funded by EC-Atlantic Region, is an adaptation of the technique described by M. Salazar (Appendix C) which will be carried out at the same location and at the same time as Dubé's mesocosm study. The second caged bivalve study, funded by DFO-Maritimes Region, will be an adaptation of the HABITRAP approach described by P. Cranford (Appendix C). In addition, a workshop is being held on 15 April 1997 at the Institute of Ocean Sciences (Sidney, BC) to discuss application of caged bivalves for monitoring mill effluents in BC. The BC government is also considering an examination of bivalves, both caged and held in shore-based tanks, for monitoring the effects of effluents from the marine mills on Vancouver Island.
Salmon mark/recapture studies: In British Columbia, the most important commercial fishery is that for Pacific salmon. It has therefore been suggested that the EEM program for pulp and paper mills discharging into rivers and the coastal environment of BC should measure impact on the salmonid resource directly. Specifically, it is suggested that juvenile salmon be exposed (either naturally or experimentally) to BKME,

tagged and released, and then returns 2 or more years later be compared with returns of an unexposed group. A similar kind of study was carried in BC to examine the impact of exposing juvenile pink salmon to oil (Birtwell et al. 1996). It is recognized that such a study would likely be considerably more expensive than a Fish Survey carried out by an individual mill. However, where several mills discharge into the same environment (e.g., the Fraser River) and their effects cannot be evaluated independently from each other, it might be more cost effective for the mills to join forces to carry out a single study that will provide relevant information of immediate relevance to the fishery.

Fish Health Assessment (suggestions from B. Antcliffe): Utility of a Fish Health Assessment as an additional endpoint will depend on the following:

1. Synthesis of Existing Data: literature review and workshop
2. Research into immunomodulation in fish exposed to BKME
3. Examine various indicators and determine which are worth measuring
4. Develop protocols for field methods: which organs and indicators; establish consistent terminology; determine baseline variation in indicators. Begin with HAI and CDS. Determine which laboratory methods are available. Determine availability of histologists.
5. Examine summary indices (HAI, CDS) and key abnormalities.
6. Examine tiered assessments: 1) gross external examination, 2) gross internal examination, 3) histology - gill, liver, kidney, spleen, hindgut
7. Age distributions should be similar between groups being compared.
8. Establish biologically important effect sizes.
9. Research into tying health effects to BKME exposure.
10. Research into cumulative effects: a) temporally; b) multiple, simultaneous impacts

vii) comparison of synoptic measurements

bivalves or invertebrate community assessments versus fish survey results:

It was the opinion of the EWG that the invertebrate and fish components of the EWG monitor different, important responses. However, comparisons of the results of the two surveys should be assessed in future cycles.

comparison of fish toxicity and fish survey results:

It was the opinion of the EWG that the toxicity and fish components of the EWG monitor different, important responses. However, comparisons of the results of the two surveys should be assessed in future cycles

The EWG recommends that further research be conducted to evaluate the implications of not detecting a change in gonad size smaller than 25%. Sensitivity analysis may be used with population models to examine the population-level consequences of the variances observed for the fish survey parameters and the target effect sizes chosen.

5.0 Recommendations

Conclusions related to review of first cycle objectives

Recommendations have been developed by the FSEWG for sentinel species, capture gear and methods, alternatives to the FS, further research needs, and interpretation of studies. The recommendations are summarized briefly in this section and are discussed in further detail throughout the report. Please see the appropriate sections for more discussion of the recommendations, and the decision tree (Section 4.2) for information on when each of the recommendations should be considered.

Sentinel Species (See Section 3.2.1.2)

The original criteria for selecting sentinel species (Munkittrick 1992) are still relevant but should not be considered final. There are other sentinel species which should be considered in some studies. **The FSEWG recommends** that alternate fish species be considered when appropriate; for example, marine and/or estuarine mills should evaluate the use of eelpout, blennies (both live-bearers) and sculpins where available and estuarine mills should consider using mummichogs (fractional spawners), killifish and sticklebacks. The information available for this review does not permit an assessment of whether sufficient numbers can be caught. Furthermore, multiple spawners and live-bearers require special attention regarding measurement of reproductive variables. If fractional spawners are to be used, the EWG recommends that they be sampled prior to initiation of the first spawning period.

Capture Gear and Methods (See Section 3.2.2)

First cycle surveys which expended effort during predesign in visiting the site and catching fish, or had recent catch data available, were generally more successful than surveys where little recent data was available. The success level of second cycle studies should increase because of the benefit of data obtained at unsuccessful sites during cycle one. The optimal method for capturing species is often site-specific, and by-catch and specificity can be optimized by adapting gear and effort on a site-specific basis. The EWG wished to note that the capture success of many species (using gill nets or electroshocking) is much higher at night than during daylight hours, and second cycle surveys can benefit in terms of capture success and reducing variability by comparing similar sites in the tables accompanying this report (Appendix III). The issue of by-catch should be discussed with the regional authorities. Information on by-catch and accidental mortalities should be recorded whenever possible, e.g. measured and reported in the EEM report. This may be especially important when too few sentinel species are caught after the minimum recommended effort has been expended.

Marine Mills:

- Trawling can be problematic in B.C. fjords because of the steep topography encountered there and is **recommended** only if problems related to depth changes and underwater debris can be overcome. Trawling may be more suitable for some bottom species (e.g. flounder, eelpout) on the Atlantic coast.
- Beach seines are **recommended** for sampling in shallow-water receiving areas. Fyke nets and traps are also suitable for sampling in Atlantic intertidal and estuary zones.
- Hook and line collections are **recommended** as a selective method for catching bottom fish such as sole.
- Hand collections are **recommended** for sedentary or slow-moving species such as rockfish, blennies and mussels.
- Gillnets are **conditionally recommended** in marine environments if they can be positioned to sample at a particular depth, and not across stratified layers of salinity/temperature. They should be single-size mesh nets. They should not be used in areas where they may become twisted by tides or currents.

- Purse seines and mid-water trawls are **conditionally recommended** for sampling fish that live in the pelagic zone. Mid-water trawls are preferable to seines because they allow more precise layers of the water column to be sampled.

Freshwater mills:

- Gill nets with a single mesh size and angling are **recommended** as selective capture methods for capturing fish in rivers and lakes.
- Trap nets and seine nets are generally less selective but, since they are non-lethal, unwanted by-catch can be returned unharmed. They are **recommended** for fishing in rivers and lakes.
- Electroshocking can be selective but care must be taken to retain only fish that will be useful to the survey. This method can be combined with other gear such as seine nets to increase capture efficiency. Electroshocking is not suitable for fish in deep or opaque water. It is **conditionally recommended** for fishing in shallow, clear water and where gill nets are not suitable.
- Purse seines and otter trawls are **conditionally recommended** as non-selective, non-lethal methods which may be useful for some species in lakes.

Reference Sites (See Section 3.2.3)

The FSEWG recommends that reference area(s) be

- as similar as possible in all respects to the exposed area except for the effluent from the mill
- immediately adjacent to or upstream of the exposure zone unless that area is unsuitable (e.g. reservoirs, etc.)
- sampled at the same or appropriate time period
- independent of Cycle One reference sites (selection of a reference site in Cycle One should not dictate its use in Cycle Two)
- described in as much detail as possible (physical, chemical and biological habitat of exposure and reference sites including measurements of temperature, depth, flow)

The FSEWG recommends that selection of reference sites should depend on

- the mobility of the sentinel species (may eliminate adjacent or immediate upstream sites as reference sites)
- the timing of sampling (fish movements may render reference sites inappropriate during some seasons)
- sentinel species present in the exposed area; there is no purpose in collecting fish from a reference area when that species was not available in sufficient numbers in the exposed area.

Areas of concern in selecting reference sites are

- presence of dams (can cause significant habitat changes which can alter the performance of some fish species)
- mobility of sentinel species
- confounding factors (e.g. other municipal or industrial outfalls); their presence should not automatically cause a rejection of the Fish Survey but the situation should be evaluated on a site-specific basis to determine the best approach
- natural variability

The FSEWG also recommends that multiple reference sites be used rather than increasing sample sizes at one site, as a means of decreasing the sample variability (see Section 3.3.2 for further detail).

The FSEWG does **not recommend** the use of a general data set of regional reference sites because

- of the difficulty of matching the receiving water characteristics of several mills within a region
- differences in the timing of sampling could have dramatic effects on interpretation
- it would generally require more reference locations than the traditional approach.

The FSEWG does **not recommend** the use of a single sample site for benthos and fish sampling because the characteristics of the fish species selected, the mobility of the fish, different habitat selection and the type of sampling gear required may make the optimal benthos sampling sites inappropriate for the Fish Survey. Although the sites may be the same in many circumstances this should not be a significant criteria for fish sample collection. However, when possible there are advantages to selecting similar sites so that the data can be used to help interpret responses.

The FSEWG does **not recommend** increased benthic monitoring, intertidal surveys (*i.e.* invertebrate or community surveys), fish community surveys, increased effluent monitoring or use of caged fish as alternatives to the Fish Survey.

Pilot Studies

The EWG recommends that pilot studies be conducted as soon as possible to evaluate the potential utility of caged bivalve studies and mesocosm studies to cost-effectively address the issue of whether there are environmental concerns at sites where mills are in compliance. If bivalve studies are shown to cost-effectively provide useful information, the EWG recommends that they be considered at sites where other alternatives are not possible but the receiving environment can be sampled and there is an effluent field which presents a potential risk. If mesocosm studies are shown to cost-effectively provide useful information at environmentally realistic concentrations, the EWG recommends that they be considered for use at sites where effluent discharge fields are a potential risk but effective field studies are not possible.

If the mesocosm studies are not shown to be cost-effective, or to provide useful information, there is considerable recent data that has shown that fathead minnow life cycle tests can be used to demonstrate impacts on egg production, maturity and secondary sex characteristics. These studies are also capable of showing that pulp mill effluent has the potential for causing negative impacts on survival and growth, and they could be considered for use if mesocosm studies would not be effective at these sites. The EWG is confident that the major risks currently known to be related to pulp mill effluent exposure (impacts on reproduction) can be detected by a fathead minnow life cycle exposure at realistic exposure levels and can indicate the potential of effluents in compliance to cause impacts. This test could be considered for those receiving environments where environmental risks are a potential but receiving environment sampling can not be conducted and mesocosm testing is not possible. The mesocosm and fathead minnow life cycle tests can also be used to isolate potential mill effects from other potential confounding factors at sites where these are a problem, but the EWG does not recommend their use at sites where the effluent field is too small to present a realistic risk of impacts when the mills are in compliance.

Cycle 2 Recommendations

If the FS in Cycle 1 was successful, or could be made successful by better capture techniques, timing sentinel selection, reference site choice AND there are no serious confounds with other effluent discharges, then proceed with a Standard FS. The sample size should be determined using the variability estimates given in Cycle 1, with a target effect size of 20 to 30% for a change in gonad size with a power of 0.8. If variability estimates are not available, a sample size of 30 males and 30 females would be conservative.

If fish were captured in the effluent field, then exposure is obvious, although the period of residency is unknown. Significant exposure is obvious in situations where large, statistically significant differences are seen between exposure and reference sites. Although these differences may not be the result of exposure to pulp mill effluents, they do indicate some significant period of residency in the effluent field, and an absence of mixing between reference and exposure fish. The main concern relates to establishing the period of residency in the area of the effluent field when no obvious barriers are present and there are no differences between sites. Most chemical and biochemical tracers only provide evidence of short-term residency (days to weeks) and they were not used very successfully in the first cycle. The real value of chemical tracers (as defined by the Tracers EWG) is to establish that reference fish did not spend significant time in the effluent field. If there is doubt about the exposure, then redesign of the survey should be considered to utilize fish which are more likely to remain longer in the effluent field.

Every effort should be made to sample two sentinel species but if only one appropriate sentinel species can be found, proceed with that one. If it is not possible or appropriate to repeat the design from Cycle 1, a modified design should be selected based on the following criteria:

a) Scientifically defensible
b) Cost-effective
c) Manageable requirements and time frames
d) Generate interpretable results
e) Provide defined decision points
f) Availability in private sector
g) Relevance to detection of adverse effects

Mills in other regions where field work was conducted prior to Cycle 1 surveys also had higher than average success. The EWG recommends that mills where success was limited initiate some focussed surveys prior to conducting Cycle 2 work. For new mills or mills which were totally unsuccessful, the EWG recommends that a reconnaissance survey be conducted prior to design of Cycle 2 fish collections.

Alternatives to the Fish Survey

In no circumstances does the EWG recommend increased benthic monitoring, intertidal surveys, fish community surveys, increased effluent monitoring or use of caged fish as alternatives to the Fish Survey.

The EWG recommends that priority should go to providing protocols for juvenile and larval fish surveys, and also to pilot testing of caged bivalves and mesocosms to evaluate their appropriateness as alternatives for Cycle 2.

Further Research Needs (See Section 4.3)

The FSEWG recommends further research in the following areas:

1. Fish biology:
   - Mobility and migration of sentinel species: local tagging studies to aid in determination of exposure
   - Optimal sampling periods with respect to reproduction and migration
   - Determining exposure of fish (*i.e.* duration of exposure of various sentinel species): consider biochemical indices, biological markers, chemistry
   - Natural variability of FS life-history variables in wild fish populations
   - Estimating reproductive potential of fractional or multiple spawners

2. Interpretation of studies:
   - Discriminating among impacts of different effluents when confounding discharges are present
   - Development of target effect sizes for growth (rather then reproduction), if juvenile fish surveys are to be used
   - Interpretation of mesocosm exposures; at least one mesocosm pilot study is planned for fall 1997 and will be used to develop protocols.
   - Interpretation of scope-for-growth studies
   - Interpretation of salmon mark-and-recapture studies
   - Interpretation of fish health assessment studies
   - Assessment of usefulness of (1) bivalve/invertebrate community surveys and (2) fish toxicity results for comparison to Fish Survey results
   - Implications of not detecting a change in gonad size smaller than 25% (sensitivity analyses used with population models to examine the population-level consequences of the variances observed for the fish survey parameters)

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APPENDIX I

ECOLOGICAL SIGNIFICANCE, EFFECT SIZE AND STATISTICAL ISSUES

prepared by

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This Appendix was written by Dr. Michael Paine of the Adult Fish Survey (AFS) Expert Working Group (EWG) in response to questions raised by EWG members in the course of their discussions. Shelley Luce, the consultant retained by the AFS EWG, provided data summaries from Cycle One studies. Any opinions, conclusions and recommendations presented below are those of Dr. Paine, and not necessarily the EWG. The main body of the AFS EWG report indicates which opinions, conclusions and recommendations were or were not accepted by the EWG.

1.0 Adult Fish Survey - Ecological Significance and Target Effect Size

This section discusses:

- the ecological significance of an Adult Fish Survey (AFS) (Section 1.1)
- the ecological significance of the target effect size (ES) proposed by the EWG (Section 1.2)

An AFS is defined as the specific type of survey conducted in Cycle One. The generic term "fish survey" refers to any type of survey which could be used intended to measure effects on fish, and includes alternatives discussed by the AFS EWG. Although the discussion of ecological significance, and especially target ES, focuses specifically on an AFS, many of the points made are applicable to alternatives.

1.1 Ecological Significance of AFS

As defined in Annex 1 (EC/DFO, 1992) and in the AFS Further Guidance Document (EC/DFO, 1995), the Cycle One AFS consisted of a field survey of demographic variables (age structure, growth, reproductive effort) measured on adults of two sentinel species, collected from reference and exposed areas. The key features of an AFS are:

- adult fish or shellfish are used as sentinel organisms
- demographic variables are used as response variables
- the survey or effects assessment is conducted in the field rather than in the laboratory

The ecological significance of each of these features is evaluated below. The evaluation also considers the important issue of whether these features and the AFS as a whole provide any important ecological information not provided by other core elements (laboratory toxicity tests; invertebrate community survey {ICS}). The evaluation addresses the important question:

Why should effects be measured on these specific variables, on adult finfish or shellfish, and in the field?

1.1.1 Adult Fish and Shellfish as Sentinel Organisms

The *Federal Fisheries Act* protects fish (finfish and shellfish) and fish habitat. The ICS addresses the issue of whether current regulations protect fish habitat. However, except for laboratory toxicity tests on early life stages, the AFS is the only element of the EEM program which assesses potential impacts on fish. Therefore, from a regulatory perspective, some type of fish survey, although not necessarily an AFS as conducted in Cycle One, is a necessary part of the EEM program. Regulatory significance does not always equal ecological significance. However, fish are also important members of aquatic communities, occupying intermediate and top trophic levels. In contrast, benthic invertebrates occupy lower trophic levels. Therefore, an EEM program without an AFS or some other fish survey would ignore an ecologically important part of the aquatic community.

An AFS focuses on adult fish because:

- reproductive effort, a key variable (see following sections), can only be measured on mature adults
- measurements of adult age structure and growth provide a record of cumulative, long-term effects over the lifetime of the fish

For practical reasons, the laboratory toxicity tests used in the EEM program are conducted on only a small portion of the life cycle, usually early life stages (ELS). The traditional justification for conducting ELS tests is that survival and growth of early life stages are the most sensitive endpoints (e.g., McKim, 1985). However, reproduction (fecundity) may be the most sensitive endpoint, and the most important variable determining contaminant effects on fish population abundance (Barnthouse et al., 1987, 1989, 1990; Suter et al., 1987; see also Section 1.1.3).

1.1.2 Demographic Variables as Response Variables

Ideally, to assess the effects of pulp mill discharges and any other anthropogenic activity (e.g., exploitation) on fish populations, fisheries biologists would use accurate and precise estimates of abundance (or biomass). In practice, accurate and precise abundance estimates are difficult or impossible to obtain at a reasonable cost. Furthermore, assessments of abundance time series are retrospective - effects are apparent only after numbers have already declined. Therefore, fisheries biologists often use other variables, such as those measured in an AFS, to assess the status of fish populations and predict future changes in abundance (Ricker, 1975; Getz and Haight, 1989; Smith, 1994).

Abundance and biomass, and their changes over time, depend on:

- age- or stage-specific survival (or its inverse, mortality)
- growth
- fecundity

Getz and Haight (1989) review the application of these variables to general population models, including those used in fisheries management; Barnthouse et al. (1987, 1989, 1990) and Power et al. (1994) provide examples of specific applications of these variables to assessment of risks from toxic contaminants to fish populations.

Fisheries biologists estimate growth and fecundity, usually adjusted for age or size, in the same way as in the AFS. Age-specific survival or mortality could be estimated from time series of abundances of single age classes or cohorts, although that requires good estimates of absolute or relative abundances. An alternative is to estimate survival from age distributions in a single or several samples, a method known as catch curve analysis (Ricker, 1975). Johnson and Landahl (1994) provide an example of the application of catch curve analysis to assessment of contaminant effects on survival of English sole (*Pleuronectes vetulus*). Theoretically, catch curve analysis could also be applied to AFS age distributions to estimate survival or mortality rates, and population rates of increase or decrease could be predicted using models incorporating those survival estimates and estimates of growth and fecundity. In practice, sample sizes from the AFS would be too small to obtain reliable survival estimates from catch curve analysis. For example, the catch curve analysis in Johnson and Landahl (1994) was based on samples of fish from four years, with >100 fish caught in each year (total catch = 1188). Therefore, age structure in the AFS should be treated as a qualitative surrogate, and not a direct quantitative measure, of potential effects on survival.

Despite the limitations of the response variables measured in the AFS, especially age structure as a surrogate for survival, those variables and the AFS still provide the best estimates of the parameters needed to assess effects on fish populations. Extrapolations of population effects from laboratory data are even more uncertain (Section 1.1.3). Any other response variables measured in a field survey must be related to survival, growth and/or fecundity to be ecologically significant (i.e., answer the "so what?" question). For example, various biochemical, histological, and other sub-organismal response variables have been proposed as appropriate for the EEM program during its evolution. These response variables may have some advantages, particularly in terms of rapid and early identification of potential effects (the same argument could be made for laboratory toxicity tests). However, proponents of these measures rarely, if ever, relate them to demographic variables and ultimately to population abundances.

In one of the few studies relating sub-organismal effects to higher (i.e., population-level) effects, Johnson and Landahl (1994) examined the relationship between liver lesions in English sole and field mortality rates in Puget Sound. Previous studies had shown that sole exposed to contaminated sediments showed a higher incidence of liver lesions and other abnormalities. These lesions could eventually be directly lethal, or could indirectly increase mortality from other sources (e.g., predation) by weakening the fish. However, field mortality rates of sole from sites with high incidences of liver abnormalities were not significantly higher than those of sole from sites with low incidences. In fact, mortality rates were *lower* for fish from sites where incidences of one type of abnormality, neoplasms, were higher. The authors discussed some limitations of their statistical analyses which might have prevented them from identifying mortality effects from liver damage, even if those effects existed. However, they also noted that any effects may have been overridden by other factors affecting mortality, such as exploitation. In particular, fishing pressure may have been lower in more contaminated areas, compensating for any mortality from liver damage. The authors also noted that even if liver damage did increase mortality rates of older females, other contaminant effects on earlier life stages and reproduction of younger females would probably be more important. Because they are few in number, older females make a relatively minor contribution to the next generation; the more numerous younger females make the major contribution. In summary, the Puget Sound study showed that high incidences of liver damage in sole did not result in reductions in survival of field populations; because liver damage tends to affect older individuals, any reductions in survival would probably have minimal consequences for abundance.

1.1.3 Laboratory Toxicity Tests versus Field Surveys

Laboratory toxicity tests have numerous advantages, which will undoubtedly be discussed in detail by the Toxicity Test EWG. Relative to the AFS, the major advantages of toxicity tests are:

- the effects of factors other than exposure can be controlled or eliminated in the laboratory; the level of exposure can also be controlled
- changes in effects (e.g., due to process changes or spills) can be rapidly identified using laboratory toxicity tests

However, these advantages can be disadvantages:

- the laboratory environment is unrealistic, and results of laboratory toxicity tests are not easily extrapolated to field populations

- toxicity tests, or at least those used in the EEM program, cannot assess long-term effects, especially on adult fish

Therefore, a comprehensive and effective monitoring program should include both laboratory toxicity tests, and an AFS or other field survey.

Section 1.1.1 discusses the importance of measuring adult endpoints, especially fecundity. Barnthouse et al. (1990) examined the uncertainties associated with extrapolating laboratory toxicity test results to field populations, building on the review of toxicity tests by Suter et al. (1987). They concluded that (p. 297, Abstract):

> "However, the quantitative effects of both factors [differences in life history between species; exploitation intensity] are negligible compared to the uncertainty introduced by estimating long-term effects from short-term tests or QSARs. Our results suggest that consideration of life history may be important for site-specific assessments. For screening-level assessments, the substantial differences in uncertainty associated with different types of [laboratory toxicity] test data are of much greater concern."

If the major source of uncertainty in assessing .population-level effects is associated with extrapolating laboratory results to the field, then the obvious solution is to measure the important variables directly in the field. What are the important variables? Barnthouse et al. (1987, 1989, 1990) proposed that reproductive potential be used as an index for quantifying responses of fish populations to contaminant exposure. Reproductive potential for a female of any age (usually age at maturity or recruitment into the fishery) is the number of females of that same age which that female is expected to produce over her lifetime. Obviously, a female must at least replace herself (i.e., reproductive potential ≥ 1) or the population will decline. Reproductive potential is calculated by combining estimates of age structure/age-specific survival, growth and fecundity, the same variables measured in the AFS (Section 1.1.2).

Note that if life history differences among species have limited effects on responses to contaminant exposure, then sentinel species selection can be based on more practical and site-specific considerations such as local abundance, ease of capture, etc. An expanded pool of potential sentinel species would then be available for Cycle Two. Barnthouse et al. (1990) examined only two finfish species, so their conclusions may not apply across the entire range of potential sentinel finfish and shellfish species. However, the two species they examined differed substantially in their life histories - one was a small (200 g), short-lived filter-feeder, and the other was a large ($\geq$20 kg), long-lived piscivore.

Finally, exploitation is one of the major potential confounding factors present in field situations. Exploitation is obviously eliminated in laboratory studies, and can also be eliminated in field studies by using unexploited species. However, even when exploitation is not controlled, the effects of different levels of exploitation are minimal compared to the effects of extrapolating toxicity test results to the field. *Therefore, what is the advantage of controlling confounding factors in the laboratory, when simply conducting the test in the laboratory is the major source of uncertainty?*

1.2 Target Effect Size(s) (ES)

The primary objective of the Cycle One AFS was to obtain estimates of the variability in the demographic parameters measured (EC/DFO, 1995). These estimates of variability would then be used to calculate the sample sizes required in the Second and subsequent cycles to detect ecologically significant effects. Obviously, an ecologically significant effect must be defined before required sample sizes can be calculated.

Keough and Mapstone (1995) discuss some general considerations for developing ecologically significant effect sizes, and provides some examples for programs monitoring the effects of pulp and paper mills. Their examples focus mostly on plants, not fish, and were taken from Australian studies. Barnthouse et al. (1989) argue that a 10% change in variables such as those measured in the AFS would be societally and ecologically significant, although they were concerned primarily with laboratory toxicity tests and not field surveys. Their proposed effect size was deliberately conservative (small) because of concerns about the uncertainty in extrapolating laboratory results to the field (Section 1.1.3).

In the absence of any strong guidance on the magnitude of ecologically significant effect sizes from the literature, the AFS EWG proposed that a ±25% difference in gonad weight relative to body weight or length (=relative gonad size) between reference and exposed areas be adopted as a target ES. The proposed ES was developed partly to expedite evaluation of Cycle One results and statistical power calculations for Cycle Two. The major advantages of the proposed target ES are that:

- it is based on the most important/sensitive variable, reproduction
- it is probably superior to most alternatives
- it is almost certainly superior to the *de facto* alternative of "doing nothing" (not defining an ecologically significant ES or not conducting the AFS only because consensus on an ES cannot be achieved)

Reproduction is probably the key parameter in the AFS (see also Section 1.1.1). Effects of pulp mill effluents on reproduction have previously been measured in field surveys (e.g., Munkittrick et al., 1994) and in full and partial life-cycle toxicity laboratory toxicity tests. Related effects on age structure/survival and growth were also observed, but effects on reproduction were generally the most severe and/or occurred at lower effluent concentrations. These studies also showed that reproductive and other effects were absent below some mills, or only occurred at high concentrations of some effluents, suggesting that site-specific factors (dilution, process, treatment) affect the response. Clearly, then, there is a need to determine the current extent of reproductive effects in Cycle Two, given recent improvements in process and treatment since the cited studies were conducted.

The 25% difference chosen for the target ES should be regarded as approximate; any percentage between 20-30% would suffice. In fact, excessive precision should be avoided when specifying target ES and calculating sample sizes for the Second and subsequent cycles (Section 2.2). There are two major problems with using smaller ES (e.g., <20% and certainly ≤10%). First, variances of reproductive parameters, even after adjustment for weight or length, from successful Cycle One studies were generally too high to provide sufficient power to detect small effects without prohibitively large sample sizes (Section 2.2). As target ES become smaller, and required sample sizes larger, the effects of sampling mortality on the population will eventually exceed any population-level effects from reproductive effects. Second, differences of <20% may occur naturally between any two unexposed and relatively similar areas (Section 2.3). If the target ES is too small, then the risks of false positives (detecting a natural difference between areas and attributing it to the mill effluent) increase.

The proposed ES of 25% was chosen partly because it corresponds with the effect size observed at several Canadian mills. Swedish studies have identified larger effects from past pulp mill discharges, although recent studies have indicated. Both the Canadian and Swedish governments reacted to these and other effects by passing tougher regulations, suggesting that reproductive effects >25% were societally or politically unacceptable. However, it is not clear how large a role reproductive effects, and the ecological consequences of those effects, played in stimulating these regulatory decisions. For some mills, treatment and process improvements made in response to regulations and public concerns reduced reproductive effects from ≥25% to <25%. Therefore, it is reasonable to expect ES<25% below modernized mills adhering to current regulations. If one chooses a higher target ES (e.g., 50%), then it becomes more difficult to evaluate the effectiveness of the regulations (the objective of the EEM program), because effect sizes below many mills prior to recent improvements may already have been <50%.

Reproductive variables are usually as variable, or more variable on a relative scale (i.e., as CV or the variance of log-transformed values) than other variables measured in the AFS (Suter et al., 1987; Section 2.1). If effect sizes are also expressed on a relative scale (i.e., as % differences), then any study which can detect a ±25% difference in relative gonad size can detect similar or smaller differences in other parameters. However, the ES which can be detected given proposed Cycle Two sample sizes should be calculated for all variables, and not just relative gonad weight. Also, the same approach used to identify a target ES for relative gonad weight should be applied to other variables. Finally, sensitivity analyses using population models should be used to explore the consequences of the ES chosen for any and all variables.

2.0 Variability of AFS Parameters

2.1 Estimates of Variance from Cycle One

Table 1 summarizes variances of the AFS parameters from Cycle One for white suckers, the most commonly used sentinel species. These variances were given in reports as Mean Square Errors (MSE) from ANOVA analyzing log-transformed ages, and ANCOVA analyzing log-transformed regressions for other parameters. To approximate Coefficients of Variation (CV) for untransformed or arithmetic values, values of 100 × ({antilog RMSE} - 1) are given in Table 1 (RMSE = √MSE). The equivalence between CV and 100 × ({antilog RMSE} - 1) breaks downs at CV>50% or antilog RMSE>1.5; values beyond those limits should simply be considered indicative of high variability. EC/DFO (1995; pp. 28-29) provides a good summary of the use and interpretation of variances of log-transformed values. Table 1 includes studies with ≥10 fish per sex per area, and only those studies which reported MSE for log-transformed data from ANOVA or ANCOVA. Some studies which included the required data may have been omitted, because the consultant preparing the summaries was not always provided with complete reports, including raw data and appendices. The EWG should expand this summary to include other species, and any results and conclusions presented here should be regarded as suggestive rather than definitive.

Table 1 indicates that variances for reproduction (either relative gonad weight or fecundity) are greater than those for growth in length and condition, and similar to those for age, growth in weight, and relative liver weight. Differences between the sexes within the same study were relatively small compared to differences between studies. Ranges of variance for any parameter among studies were broad, suggesting that variances could be reduced in many studies (see Section 2.2). Most medians were much closer to minima than to maxima, partly because antilog RMSE were log-normally distributed. However, there were also some apparent outliers - studies with unusually high variances for most or all parameters. Table 2 provides Spearman rank correlations among RMSE for the AFS parameters. All correlations were positive, again indicating that studies with high variance for one parameter usually had high variances for other parameters.

Table 1 excluded many Cycle One studies, which could provide potentially useable data. To expand the database for one variable, relative gonad weight, Table 3 summarizes CV for reference area GSI for several commonly used sentinel species. Any study reporting a standard deviation or CV for GSI for ≥10 reference fish of a sex was included. GSI and its SD or CV were reported more frequently than MSE for log-transformed values, and GSI is a measure of relative gonad weight, the parameter chosen for the target ES.

Table 3 reinforces and adds to several conclusions from Table 1:

- CV for the two sucker species (longnose and white) were similar
- data for comparison of other species with suckers were sparse, but most had higher CV than suckers
- there was a broad range of CV within species (i.e., among studies)

These GSI CV were used for power calculations in Section 2.2.

2.2 Power Analyses

2.2.1 Power Analyses for Cycle Two AFS

Figure 1 shows the effect size (ES), expressed as the % difference between exposed and reference fish, which can be detected with sample sizes of 20 fish per area (i.e., as in Cycle One). The ES were calculated using methods described in Green (1989) and on p. 27 of the Technical Guidance Document (EC/DFO, 1993), assuming a 2-tail t test with $\alpha=0.05$. Two lines are given, one for power $(P;=1-\beta)=0.80$ (i.e., 80% probability of detecting the ES) and one for $P=0.95$. The range of CV on the X axis corresponds to the range of CV for sucker GSI in Table 3. Ideally, calculations should be made using the $\sqrt{MSE}$ for log-transformed values in Table 1, following procedures in EC/DFO (1995; pp. 28-32). In practice, using CV for GSI will provide approximately the same power relationships, and is somewhat simpler for presentation purposes. Furthermore, all power relationships presented are generally applicable if ES and CV are converted to SD units.

The two relationships in Figure 1 are straight lines, because for any P, and fixed n, the ES which can be detected is a direct function of CV. For $P=0.95$, ES=1.18(CV); for $P=0.80$, ES=0.91(CV). Therefore, minimum sample sizes in Cycle One (i.e., $n=20$) were adequate to provide reasonable power (i.e., $P\approx0.90$) to detect ES of one SD, regardless of whether ES and SD are expressed in original units, as CV, or as $\sqrt{MSE}$ for log-transformed values. It follows that any Cycle One study with $n\geq20$ and CV for GSI $\leq25\%$ was powerful enough to detect the target ES. These studies need only be repeated in Cycle Two to confirm that the Cycle One results are consistent over time, and to correct any problems with other aspects of the study.

Figure 2 provides sample sizes required to detect an ES of 25% for various CV, again for $P=0.95$ and 0.80. Figures 2 through 5 were based on tables in Cohen (1988). Because n is approximately proportional to the square of CV, required sample sizes increase rapidly for higher CV. If we assume that the required n can be collected over several cycles (see Section 2.3), with $n=20$-30 fish per cycle, 2-5 cycles would be adequate for CV$\leq$50% to provide $P=0.95$. That would fit within a 20-year time-frame for the EEM program, which is probably the maximum currently contemplated. Higher CV would be adequate if $P=0.80$ were used, but a higher P may be preferable, given that some unforeseen complications may arise in analyses of multiple-cycle studies (Section 2.3). Therefore, studies with CV>50% may not provide adequate power unless sample sizes are increased from those in Cycle One. The best approach in these studies would be to reduce CV, because of the square relationship between n and CV. For example, reducing CV by 20% (e.g., from 25% to 20%) reduces required n by ~36% ($0.8^2=0.64$), or more than one-third.

Figure 3 provides required n to detect various ES, given CV of 25% (the approximate average for sucker GSI). Because n is proportional to $(1/\mathrm{ES})^2$, required sample sizes escalate rapidly for detection of effects <0.8×CV (i.e., <20%), and especially 0.5×CV (i.e., <12.5%). Alternatively, increasing n beyond 20-30, and certainly beyond 50, produces rapidly diminishing returns in terms of reducing the ES which can be detected. Given the CV in Table 3, setting a target ES <20% would require prohibitively large sample sizes and/or more cycles than currently contemplated.More generally, in any study in which there are significant costs associated with processing additional replicates, it is not cost-effective to attempt to detect ES much less than one CV or SD (or their log equivalents). Again, the solution is not to increase sample sizes, but to reduce variances, which effectively shifts the curves in Figure 3 to the left. This is shown in Figure 4, where n versus ES is compared for CV of 20% and 30%, for $P=0.95$.

Figure 2 through 4 span such a wide range of CV, ES and especially n that it is impossible to evaluate the effects of small departures from minimum required Cycle One sample sizes (e.g., 15 or 30 fish per area versus 20), and the power associated with $n\leq20$. Figure 5 provides the ES which can be detected for sample sizes ≤30. The ES is given in dimensionless units, as a multiple of SD or CV (or their equivalents for log-transformed values), so the figure is universally applicable. Substantial gains in the ES which can be detected can be achieved with increased n for $n\leq10$. Significant gains can still be made up to $n=20$. There is little difference in the size of ES which can be detected for $n>20$, again illustrating that increased sample sizes produce ever-diminishing returns in terms of increased power. It follows then that the definition of successful ES as those in which ≥20 fish per sex per species per area were caught could be relaxed to include cases in which ≥15 or even ≥10 fish were caught, especially when one considers combined sample sizes from several cycles.

The steep decline in detectable ES with increasing *n* at small *n* is a function of increased confidence in the estimate of variance (see Appendix B of EC/DFO, 1995). At *n*>20 or even >10, confidence limits (CL) for variance estimates do not shrink appreciably, and any increase in power comes from narrowing CL limits for the means, which in turn depend on $\sqrt{n}$. In other words, CL for both means and variances decrease substantially with increasing *n* for *n*≤20 and especially *n*≤10, whereas any decreases in power for increasing *n* beyond 20 come almost exclusively from narrower CL for means only. Therefore, the region *n*=0-20 is the most important for achieving increased power with increased sample sizes.

Sample sizes <10 should probably be avoided, because of potential problems associated with limited ranges for *X* variables, particularly age, in ANCOVA. Also, the capture and processing costs of small increases in sample size (e.g., from <10 to 15-20) are minor relative to base costs for travel, mobilization, boat rental, etc., which are independent of sample size. Note the contrast with laboratory studies, in which base costs are small and/or amortized over many tests, and costs associated with set-up, daily monitoring, and tear-down, and processing of individual replicates can be high. For that reason, laboratory tests often use *n*≤5, which compromises power (Figure 5; Section 2.2.4).

In summary, some Cycle One studies, even some which were unsuccessful in terms of achieving minimum sample size requirements, will have sufficient power to detect the target ES of a 25% change in relative gonad weight if repeated with little change in future cycles. Other studies with high variances for reproductive and other variables may not have adequate power. Only limited gains in power can be achieved in these studies by increasing sample sizes within cycles to *n*>20 or increasing the number of cycles. Instead, these studies should focus on reducing variances; if that fails, alternatives should be considered.

2.2.2 Practical Considerations for Power Calculations

Section 1.2 argues that the target ES of 25% for reproduction should be regarded as a range (e.g., 20-30%) rather than a precise value. More generally, a target ES for any variable should be regarded as approximate, and the sample sizes chosen for future cycles should reflect a balance between what is desirable and what is achievable given various practical considerations. There are real risks associated with detecting any precisely defined ES because required *n* is very sensitive to small changes in the other power variables (ES, CV or SD, *P*).

To illustrate the dangers of excessive precision, consider a hypothetical case in which an investigator has surveyed two species in Cycle One, one with CV=20% for GSI and the other with CV=30%. Ideally, in power calculations, the variance used should be the actual population variance rather than an estimate based on a relatively small sample. Any variance estimated from a sample will have some uncertainty associated with it. For example, in our hypothetical case with two species, the two estimates of CV are not in fact significantly different at α=0.05 or even α=0.20 (Sokal and Rohlf {1981, pp. 189-90} provide the test used; it is better to compare variances of log-transformed values rather than CV to eliminate the effects of uncertainty about the mean as well as SD in CV). Therefore, the investigator should have little confidence that the same species differences in CV will exist in future cycles, especially when it cannot be assumed that the true population variances will not also change over time. However, if sample sizes for any ES are calculated for the two species, *n* for CV=30% will be approximately double those for CV=20% (i.e., $n \propto CV^2$; $\{30/20\}^2=2.25$). It would be better to take a more realistic approach, and assume that:

- CV for both species are similar
- an ES of a 20-30% change in relative gonad weight can be detected with $P \approx 0.80-0.95$ for both species with n=20, as in Cycle One

It follows from the above, and from Section 2.2.1, that sample sizes required for detecting an ES of 20% would be approximately double those for detecting an ES of 30%, yet there is arguably little difference in ecological significance between the two ES (Section 1.2). The value of *P* also affects *n*. For example, *n* for *P*=0.95 and fixed ES or CV are approximately 50% greater than those for *P*=0.80 at small sample sizes (e.g., <20), and the difference increases with increasing sample size. However, the difference in ES which can be detected for fixed *n* between the two power levels in Figure 1 is ≈30%, which is relatively small (e.g., smaller than the difference between ES of 20 versus 30%).

In conclusion, it is dangerous to calculate *n* from other parameters, because of uncertainties about the true population CV, the power required, and the ES which is ecologically significant. It is much better to calculate CV, ES or *P* for some reasonable range of *n* such as 10-30 for the AFS in a qualitative sensitivity analysis. This type of approach usually reveals that there is little to be gained by increasing sample sizes beyond 20, and that the most cost-effective way to improve power in most studies is to reduce variances (i.e., as concluded in Section 2.21). After the study has been conducted, the confidence limits for the difference between reference and exposed areas should be calculated and reported even if the difference is not statistically significant (Paine et al., 1996).

2.2.3 Reducing Variances

2.2.3.1 General Comments

Given that reducing variances is often the best means of increasing power, how can variances for AFS variables be reduced? For the studies in Table 1, capture method appeared to influence variance. The highest variances for most parameters came from studies in which fish were captured using variable mesh gillnets and electroshockers. These capture methods are less size-selective than fixed mesh gillnets, and variances for age were often high when these methods were used. There was some suspicion that immature fish, or at least fish which would not spawn in the upcoming spawning season were being included in statistical analyses. Inclusion of immatures will obviously inflate variances for reproductive parameters. However, immatures also have different growth rates than mature adults, which devote relatively more energy to reproduction than to growth. Consultants conducting Cycle Two AFS should be clear that this is an *Adult* Fish Survey, and that immatures should be excluded. Most consultants were aware of that, and excluded immatures when analyzing fish caught with variable mesh gillnets or electroshockers (see Section 2.2.3.2).

Once only adult fish are selected, variances for age cannot easily be reduced because those variance are a function of the adult lifespan of the species (i.e., SD is related to range), which is beyond investigator control. Some minor reductions in variance may be achieved by careful QA/QC for ageing procedures. Variances for growth in weight or length are usually small relative to those for other variables (Table 1), and much of the variance may come from measurement and other error for the X variable (age) rather than the Y variable (weight or length) (Ricker, 1975). Variances for condition (i.e., regressions of weight on length) are usually low because both variables are measured with little error.

Variances for relative liver weight are high because the liver can be difficult to remove completely from suckers and other cypriniforms (minnows, carp, etc.) with diffuse livers. The experience of several EWG members is that the livers are easier to remove from these species when carcasses are partially frozen. Certainly, substantial error is introduced when carcasses are not dissected or frozen rapidly on warm days; the digestive enzymes released from the gut digest the livers rapidly. Finally, reductions in variance may be achieved by using dry rather than wet weight, although drying can be difficult in the field.

Finally, several EWG members noted that variances for relative measures (i.e., parameters analyzed in ANCOVA) depended partly on the covariate used. For example, regressions of organ (liver, gonad) weight on length might be less variable than regressions on weight. There are also several possible length or weight variables which could be used. Age could also be used as an additional covariate for analyses of ovary weights (Section 2.3). No systematic attempt was made to evaluate covariates; the author's experience is that the choice of covariates has little effect on variances. However, that may not be true in other studies, and differences in variance and power among covariates can easily be explored in any study. When using body weight as a covariate, especially for analyses of gonad weights, one must be careful that body weight excludes gonad weight. Otherwise, regressions of gonad weight on body weight are regressions of a part on the whole, and spurious correlations will be induced.

2.2.3.2 Variance of GSI

Given the broad range of CV for GSI, a key variable, even within species (Table 3), the greatest gains in power can be achieved by reducing variance for reproductive parameters. Three factors which might influence CV of female white sucker GSI were explored: capture method, sample time, and duration of sampling.

Capture Method

Some of the highest variances in Table 1 came from studies in which variable mesh gillnets or electroshockers were used. However, Figure 6 indicates that capture method had little influence on variance of GSI for the somewhat different set of studies used for Table 3. Figure 6 excludes the studies in Table 1 with the highest variances, as those studies generally did not report GSI and its SD (or the data were not provided to the EWG consultant). Even if immatures were collected by variable mesh gillnets or electroshockers, consultants conducting the studies in Figure 6 obviously culled those immature prior to calculation of GSI. With one exception, all CV were <50%, and would probably not need to be reduced much to provide sufficient power for subsequent cycles. Finally, the number of studies in Figure 6 is greater than the 25 reported in Table 3 because two methods were used in several studies. Those studies appear as an observation for each method used.

If consultants are careful to cull immatures, almost any capture method should provide suitable CV for GSI, at least for white sucker females. The major objection that the EWG had about the use of variable mesh gillnets was that bycatch mortality was often unacceptably high. Bycatch mortality will also be high for electroshocking, if all fish caught are later sacrificed.

Sample Time and Duration

Several EWG members thought that variances for GSI might be reduced if sampling occurred closer to spawning time, which for suckers would mean later in fall (assuming some migration to spawning sites in spring). Variances might also be reduced if sampling was concentrated within a short time, since gonads will mature (increase in weight) over time. However, Figures 7 and 8 show that CV for female white sucker GSI do not appear to decrease later in the year, and are not greater for longer sampling durations. There are some problems with these graphs, because they mix studies from a broad geographic range. However, Dr. Munkittrick of the EWG was able to provide some data from a single study site which showed that CV for GSI did not decrease if sampling occurred later. Sampling durations in Figure 8 may be overestimated because the sampling times given in reports may have applied to several areas, whereas the GSI apply only to the reference area (i.e., sampling times for the reference area only were rarely provided). In general, consultants conducting AFS should minimize the duration of sampling, regardless of effects on GSI within areas, to avoid confounding time effects with area effects, and to limit costs.

As noted in Section 2.2.3.1, one factor which would inflate CV for GSI and other reproductive parameters is inclusion of immatures in estimates of CV. Obviously immature fish should be excluded from analyses of reproduction, but sometimes maturity can be difficult to evaluate. Sexual maturity is much easier to determine if fish are sampled later in the year, closer to spawning time. This was a problem in marine studies, because the gonads of some species apparently do not mature until just before spawning, by which time the fish may be migrating to spawning grounds.

Other Factors

Problems may also occur when fish spawn more than one clutch in a year, or skip reproductive seasons. Many species of small fish produce multiple clutches over protracted spawning seasons (e.g., Heins and Rabito, 1986; Paine, 1990). Samples of females collected during the spawning season will contain individuals which differ in time since spawning their last clutch, inflating CV. Even if the fish are captured before spawning, the gonad mass may represent a small fraction of the reproductive output for the year (i.e., GSI are biased estimates of reproductive effort, and the bias may differ among females, inflating variances). Evidence from one group of small fishes, darters, suggests that guarding species may be less likely to produce multiple clutches, possibly because of limitations on the total length of time that a male can spend guarding a nest (Paine, 1990).

Individuals which skip reproductive seasons can usually be identified as negative outliers from ANCOVA. In other words, plots of residuals from ANCOVA will be skewed left, and will not be normally distributed. Residual diagnostic procedures outlined in EC/DFO (1995) and in Tabachnik and Fidell (1987) will usually identify if some individuals are skipping reproductive years. These individuals should be excluded from analyses of reproductive, and possibly all, variables. The reduction in variance achieved will usually more than compensate for any loss of power from reduced sample sizes. If females skipping reproductive years are excluded, that exclusion must be made objectively, and not by excluding individuals until the difference or CV the investigator wants is obtained. Also, the frequency of such individuals in reference versus exposed areas should be provided, in case skipping reproductive years is related to exposure. Obviously, sample sizes from AFS will never be adequate to test whether the relatively low incidences of females skipping reproductive years differ between areas (Cohen, 1988). However, that potential problem is no different from the problems associated with deciding whether to include or exclude dead individuals when examining differences in reproduction or growth in laboratory toxicity tests.

Figure 9 provides an example from the Fibreco Pulp Cycle One EEM report (EVS, 1996a). Females for which ova could not be differentiated during ovum counts (squares) were defined as females which would not spawn in the fall following the late August sample collection, and eliminated from analyses. It might have been better to exclude all females with ovary weight ≤2 g, since two of the females with "undifferentiated ova" had relatively large ovaries which may have been poorly preserved. However, the procedure adopted was more objective, since the ovum counts were made by someone other than the data analyst, and analyses were expedited because cases with missing values for ovum weight could simply be excluded. Removing the females with undifferentiated ova reduced overall sample sizes from 60 to 48, but also reduced antilog RMSE for log-transformed ovary weight versus body weight ANCOVA from 2.4 to 1.6 and normalized the distributions of residuals within areas. Similar reductions in variance were achieved by excluding female longnose suckers with undifferentiated ova in the Slave Lake Pulp study discussed in Section 2.3. Longnose suckers from the Fibreco study did not appear to skip reproductive years, suggesting that the phenomenon may be site-specific. Unfortunately, as Figure 9 also shows, it is much more difficult to identify males which might skip reproductive years, if in fact that ever occurs.

Unfortunately, it was impossible to estimate the degree to which production of multiple clutches by small fish and skipping reproductive years by large fish may have inflated variance for GSI in Cycle One studies. Suspicious cases could theoretically be identified by examining residual plots, but that would be better done by the consultants conducting the studies, and regulators reviewing them. More generally, any more extensive analyses of Cycle One data should focus on factors which may affect variances, and further guidance for the AFS should identify methods to remove the effects of these factors in subsequent cycles.

2.2.4 Power for AFS versus Laboratory Toxicity Tests

Finally, what is the power of field surveys, and specifically AFS, relative to laboratory toxicity tests? Table 4 provides average ES at MATC (Maximum Acceptable Toxic Concentrations) from a review of laboratory fish toxicity tests (Suter et al., 1987). The MATC are geometric means of the NOEC (No Observed Effect Concentrations; the highest concentration at which significant differences from controls were not observed) and LOEC (Lowest Observed Effect Concentrations; the lowest concentration at which significant differences from controls were not observed). Therefore, the ES given are roughly equivalent to detectable ES for P=0.5.

Table 4 also includes estimates of the CV or antilog $\sqrt{MSE}$ for log-transformed values which would be required in an AFS with n=22 to achieve the same power as the "average" toxicity test. With n=22, an AFS can detect a difference of 0.5 SD with P=0.5, and 1-tail α=0.05 (Cohen, 1988); 1-tail tests for reductions in the variables listed were used in the laboratory tests. A sample size of 22 was chosen for convenience in calculations; required variances for an AFS with n=20 would be slightly lower. For any variable, an AFS will be more powerful than a laboratory test if the field variances are lower than the CV or antilog RMSE given in Table 4.

Weight as measured in laboratory tests is usually growth, provided that initial weight is either subtracted or is small relative to final weight. Based on Table 1, the "average" (i.e. median) AFS using white suckers could detect growth effects similar to those detected by laboratory toxicity tests. However, laboratory toxicity tests usually focus on effects on growth of younger individuals, which is more rapid than growth of older individuals. Therefore, the actual growth effects which occur in laboratory studies may be greater in laboratory studies than in field surveys (i.e., observed ES/CV or SD may be more comparable).

Laboratory tests of effects on fecundity and ovum weight, the two components of ovary weight, do not have much power, since the average ES were 42 and 35%, respectively. Most Cycle One AFS with n>20 or even n>10 would have greater power to detect similar ES for relative gonad weight. However, fecundity and ovum size are usually negatively correlated, so their product (ovary weight) may have lower variance than either. Thus, the ES for ovary weight at the MATC in laboratory might be, e.g., 30%. In that case, AFS with n=22 and CV for GSI>60% would be less powerful than the "average" laboratory test.

Comparisons of power for growth and reproductive variables between laboratory and field studies are arguably unfair because covariates are rarely used in laboratory studies but are commonly used in field studies. For example, weight is adjusted for age in AFS, and reproductive parameters are adjusted for body size. However, in laboratory tests, growth or weight is usually measured on fish of the same age, and reproduction is usually measured on fish of the same size and age, so that age and size effects are effectively removed by design rather than by use of ANCOVA.

Table 4 was not really intended to show that AFS are more powerful than laboratory toxicity tests, which is partly a comparison of "apples and oranges" anyway. Also, the power of laboratory tests for detection of effects on, e.g., larval or adult survival is probably much greater than the power of field surveys which calculate survival from abundance estimates. Instead, Table 4 was provided to show that it is almost impossible to defend the opposite position that laboratory tests are more powerful than the AFS and other field surveys, a common misperception. As stated in Section 1.1.3, both laboratory and field studies have their pros and cons and both should be included in any EEM program. Furthermore, the broad range of ES at the MATC for any variable in laboratory studies in Table 4, and the broad ranges of variances for the AFS provided in Tables 1 and 3, clearly indicate that power varies considerably within both the laboratory and the field. In both types of studies, the focus should be on reducing variances, although laboratory studies would also benefit considerably from increased replication up to n=10 or 20.

2.3 Higher-order Variances

Sections 2.1 and 2.2 focus on estimates of variance among fish within areas, since those were generally the only estimates available from Cycle One studies. In a multi-cycle EEM program, industry, regulators and consultants must also consider higher-order variances. If AFS (or any other studies) are conducted over several cycles, the issue of whether differences between reference and exposed areas, or more generally, ES, are stable over time must be addressed (=spatial-temporal variance). Naturally occurring differences between similar unexposed areas can be used to set a lower limit for target ES (Section 1.2) (=natural spatial variance). If results for one or more cycles are compared regionally or nationally to assess the effects of differences in treatment or process, then the variance of differences between reference and exposed areas among similar mills must be addressed (=variance among mills).

2.3.1 Natural Spatial Variance

Table 5 provides a summary of the natural spatial differences observed for AFS variables in Cycle One studies. The summary is based on studies in which multiple reference areas were used, studies in which pre-operational data were available for reference and exposed areas, and studies in which a currently unexposed area intended to be the site of a future discharge was sampled. If target ES are to be defined as greater than natural differences, more data on natural differences are required than are presented in Table 5.

In the Slave Lake Pulp (SLPC) study, discussed in more detail in Section 2.3.2, there was a strong habitat gradient down the Lesser Slave River, which accounts for most of the differences among areas. The consultants conducting the SLPC AFS (and ICS) were aware of the gradients in the Lesser Slave River and assumed *a priori* that natural spatial differences existed. A BACI (Before-After; Control-Impact) design was used. Reference (=Control) and Exposed (=Impact) areas were monitored both before and after the mill began operation. Impacts were defined as changes in the difference between Reference and Exposed areas after operation or discharge started (Green, 1979). Thus, natural spatial differences were expected, but irrelevant for impact assessment because of the BACI design used. Even with the strong natural gradient on the Lesser Slave River, most of the pre-operational differences were small (≤10%) for white sucker (Table 5; EVS, 1996b). For longnose suckers, there were some large differences for age, growth in weight, and liver weight. For both species, differences in relative gonad weight were below the target ES of 25% (differences for female longnose suckers could not be calculated because slopes of regressions of ovary weight on body size differed among areas and years).

2.3.2 Spatial-temporal Variance and Multi-cycle Studies

In a multi-cycle AFS, it is not unreasonable to expect some temporal variation of any natural differences between areas. A real effect from a mill discharge might also vary because, e.g., effluent dilution varies among cycles. This section considers mostly natural spatial-temporal variance which might be large enough to create problems with false positives. The emphasis was on natural variance because there were arguably no mill effects in the example study used, the 1989-94 SLPC AFS. Therefore, the temporal variance of real effects could not be examined in detail. Results from a multi-year study conducted by Dr. Munkittrick of the EWG indicate that apparent mill impacts on reproductive and other parameters are likely to change in magnitude, but not direction, over time.

There are some potential statistical problems associated with repeating the AFS over several cycles. There are two factors - Area and Cycle. Unless investigators are specifically interested in what happens in each Cycle year (i.e., are interested in 1999 but not some other potential sample year such as 1998 or 2000), Cycle should be treated as a random, and not a fixed factor. Then differences among areas are tested against the MS for the interaction between Area and Cycle (A × C), and not against the variance among fish within areas. The design is effectively a paired *t*-test, with area means paired within each cycle. The A × C term is a measure of the variance of differences between areas over time. Cycles, and not fish, are replicates (actually, blocks), and there will be limited numbers of cycles and little power.

The A × C interaction can be pooled with the variance among fish, and differences among areas against the pooled error, if the interaction is small (e.g., $p>0.20$ or 0.25). This is a fairly common ruse to increase power, and is accepted by many statisticians (e.g., Winer, 1971). More recently, Underwood (1993) argued that interaction terms like A × C should be pooled with lower-order variances (e.g., among fish within areas) even when $0.05<p<0.25$ to guard against Type II errors (i.e., false negatives, or not detecting an impact when one exists). The problem with that approach is that the risk of Type I errors, or false positives, may increase. Finally, Sokal and Rohlf (1981) take the opposite position and state that a higher-order term should never be pooled with a lower-order term, unless the variance for the higher-order term is suspiciously *small* and based on few degrees of freedom (df). Their approach guards against the risk of making a Type I error when using suspiciously small higher-order variances, and is probably suitable for pure science where Type II errors may be of less concern. However, in applied science and impact assessment, the consequences of Type II errors, as well as Type I errors, are of concern (e.g., Peterman, 1990; Underwood, 1993), and the position of Winer (1971) or even Underwood (1993) should be adopted for the EEM program.

There is a paradox associated with multi-cycle studies and the A × C interaction, which may not be obvious. If sample sizes within cycles are increased or variances within areas are reduced (both commendable), the power to detect a difference between areas increases within any cycle. However, over several cycles, the power to detect temporal changes in the differences between areas (i.e., A × C interactions) also increases. Thus, investigators would be more likely to find that, e.g., $p<0.20$ for the interaction, which may then reduce overall power to detect a consistent effect (i.e., difference between areas) in a multi-cycle analysis. Consultants analyzing multi-cycle AFS data should be aware of this paradox, and avoid over-interpretation of statistically significant but small A × C interactions and differences between areas.

Tables 6 and 7 provide a summary of results for the two sentinel species used in the SLPC AFS. Fish were sampled annually in fall from 1989 to 1994, except in 1993, from four areas, one reference (R) above the mill, and three exposed areas below the mill (E1-3). The mill began operating in late 1990, so that 1989 and 1990 are pre-operational years, and 1991-94 post-operational years. Because of limited catches in some years and areas, analyses of white suckers were limited to the R and E1 areas, and 1991 data were omitted for females and 1989 and 1991 data omitted for males. All years were included for analyses of longnose suckers, but E3 was not included because that area could not be sampled in 1992 because of limited access due to low water levels. Finally, sample sizes within areas in any one year were often <20, and in a few cases <10 for males. However, total sample sizes were ≈150 for white sucker females and both sexes of longnose suckers, and ≈80 for white sucker males.

EVS (1996b) provides details of the statistical model used for analyses of the SLPC AFS data. One of the terms in the model, Y{BA} × L (Y{BA}=years within pre- {=Before} and post-operational {=After} periods; L=location or area), provided a test of the variance of area differences over time. This term can be considered natural spatial-temporal variance, since there was little or no evidence that the differences between areas reflected mill effects (see below). The Y{BA} × L term does not include changes in the differences between areas between pre- and post-operational years (i.e., BA × L), because the test of BA × L was the test for impacts.

Table 6 summarizes results of tests of the Y{BA} × L term for all AFS variables; there were two more variables for females than for males because fecundity and ovum weight, as well as gonad weight, were analyzed. Relative gonad weight was not analyzed for longnose females because regression slopes differed significantly among areas and/or years. The total number of cases in which Y{BA} × L was significant at $p \leq 0.05$ (6/27 or 22%) or $p \leq 0.20$ (12/27 or 44%) was greater than expected by chance alone. Therefore, there was added spatial-temporal variance, again presumably natural. This added variance was greater for longnose suckers, as the only cases in which Y{BA} × L was significant at $p \leq 0.05$ occurred for this species. As expected, Y{BA} × L tended to be more significant for growth and condition, the variables with the lowest variance within areas. However, some of the most significant interactions were observed for age, which had a relatively high variance within areas. Since age structure should not really change rapidly over time unless there is catastrophic mortality, analyses of age may be problematic in multi-cycle AFS. The variance of age differences between areas over time in the SLPC study was probably a function of the small sample sizes used in each year, and the broad range of ages for mature adults of the two species (3-18 years).

One must be cautious about assuming that any statistically significant spatial-temporal variance in the SLPC study was large; tests of Y{BA} × L were powerful, with >100 error df except for white sucker males. Table 7 summarizes temporal changes in area differences in relative gonad weight (relative fecundity for longnose sucker females). Except for white sucker females ($0.05 < p < 0.20$), p values for Y{BA} × L were >0.5, indicating that there was *no* added spatial-temporal variance for these reproductive variables in three of four species-sex combinations. Furthermore, there were no differences between areas >20%, and only 5 of 17 were >10%. Within either the pre-operational or post-operational periods, no temporal changes in area differences were >20%; of 11 possible pairwise comparisons, only 3 changes in area differences were >10%. Collectively, these results suggest that natural spatial and spatial-temporal variance will not lead to false positives for reproductive effects, provided that investigators focus on effects >20%. Alternatively, the results could be used to support the argument that the risks of false positives would be unacceptable if the target ES were set at <20% and especially <10%. Of course, comparable data from other mills is required before these conclusions can be generalized.

Table 7 also includes values for "Post vs Pre", which are changes in average area differences from pre- to post-operational years (i.e., BA × L). Following Winer (1971), BA × L can be tested against the MSE (variance among fish within areas) for both sexes of longnose suckers, since $p > 0.50$ for Y{BA} × L, the proper error term for testing BA × L. In fact, Y{BA} × L was so small for longnose suckers, and male white suckers, and based on so few df, that it might be pooled with MSE even if the stricter rules of Sokal and Rohlf (1981) were followed. BA × L was not even close to significant at $p \leq 0.05$ for either sex of longnose suckers, despite the power of the test (>100 error df). These results were not unexpected. The SLPC effluent is rapidly diluted immediately below the mill to concentrations <1%; there are no large tributaries below the mill to provide significant dilution and differences in exposure between E1 and E2. It has never been possible to demonstrate exposure differences between E1 to E3 using several different tracers; in fact, it has been difficult to demonstrate that any longnose suckers have ever been exposed to anything other than very dilute effluent concentrations. For these reasons, the consultants conducting the SLPC study recommended that the longnose sucker survey be dropped or replaced by a survey of small fish or bivalves found closer to the mill and in the reference area.

The Post versus Pre comparison is more interesting for white suckers, since exposure differences between R and E1 were evident using MFO (males) and resin acids in bile (females) as tracers. The differences in tracer values were not large, were based on limited sample sizes, and should be considered qualitative (i.e., absent above the mill; present below). BA × L tested against the MSE within areas was significant at $p<0.05$ for relative gonad weight for both sexes. That test was potentially suspect for females, since $0.05<p<0.20$ for Y{BA} × L, but the approach of Underwood (1993) was adopted. In both sexes, relative gonad weight was greater in R before mill start-up, but that difference was eliminated (females) or reversed (males) after start-up. Superficially, these changes appear to be enrichment effects; in females, they were associated with related post-operational increases in condition of exposed relative to reference fish. However, if there was an enrichment effect attributable to the mill, it was mostly relative and not absolute. Relative gonad weight for females in E1 was actually reduced after start-up, but the reduction in the reference area was much greater. In males, the "effect" consisted of an increase in relative gonad weight in E1 after start-up and a decrease of similar magnitude in R. The decrease in reproductive effort in both sexes in the reference area coincided with a 10-100-fold reduction in benthos abundance which occurred in reference but not exposed areas. The obvious interpretation was that the natural reduction in prey abundance above the mill reduced the energy resources available to suckers there, and consequently the energy devoted to reproductive effort was reduced.

The SLPC study is discussed in some detail, because it shows that there may be some added spatial-temporal variance in multi-cycle studies which may create problems for analysis or interpretation. Also, there was an apparent enrichment effect in white suckers which was mostly the result of natural changes in prey abundance in the reference area (i.e., a potential false positive). However, any problems with the study, which are mostly associated with the large overall sample sizes and high power, can be eliminated by simply treating any differences or apparent effects on relative gonad weight smaller than 20% as no greater than natural differences. The same approach could be adopted for other variables, with target ES and ES of concern set at levels greater than natural spatial or spatial-temporal differences. Unfortunately, there are few data sets available to do that. Therefore, the best that the AFS EWG can do is to recommend that the issues discussed in this section be considered carefully in analyses of multi-cycle data.

Finally, although there may be some problems associated with multi-cycle AFS, those problems pale in comparison with the potential problems associated with the ICS. In the SLPC study, the large change in differences in benthic invertebrate abundances between reference and exposed areas were discussed above. More generally, the Y{BA} × L term was large and statistically significant for almost every ICS variable in the SLPC study. The consultants conducting the study argued that years (i.e., cycles), and not samples within areas, were the appropriate units of replication for the ICS, and will never again analyze anything other than annual station totals based on pooling replicate samples within stations. The potential problems with multi-year ICS are not restricted to the SLPC study, nor are they restricted to freshwater environments. Otway et al. (1996) analyzed marine communities near Australian sewage outfalls and argued convincingly that for marine communities spatial-temporal variances are the proper error terms for tests of impacts. Support for that position comes from papers in two special issues of the Australian Journal of Ecology (Vol. 18, 1993; Vol. 20, 1995), and from a report providing recommendations for assessment of impacts from Australian bleached Kraft mills (Keough and Mapstone, 1995). Of relevance to the AFS EWG, several authors in these sources and Otway et al. (1996) also argue that spatial-temporal variance must be used for tests of impacts on fish communities. Fish community surveys have been suggested as a potential alternative to the AFS, but sample size requirements in terms of numbers of cycles may be impracticably large.

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Table 1. Variances (as 100*{antilog RMSE - 1}) of AFS variables for white suckers in Cycle One.

Variable	Sex	Number studies	100*(antilog RMSE - 1)		
			Minimum	Maximum	Median
Age	Female	18	21	190	41
	Male	15	24	183	45
Body wt. vs age	Female	10	18	97	28
	Male	13	18	112	59
Length vs age	Female	10	5	24	7
	Male	7	6	24	6
Body wt. vs. length	Female	19	7	19	11
	Male	15	7	23	12
Liver wt. vs. wt. or length	Female	19	18	59	28
	Male	11	20	79	28
Gonad wt. vs. wt. or length	Female	19	21	145	23
	Male	15	17	71	54

NOTE: summary includes studies with n>=10 from both reference and exposed areas

Table 2. Rank correlations among RMSE for AFS variables for white suckers

Females

	Age	Body wt. vs age	Length vs age	Condition	Liver wt. vs wt. or L	Gonad wt. vs wt. or L
Age		0.528	0.852	0.682	0.623	0.741
Body wt.	10		0.457	0.528	0.852	0.714
Length	8	8		0.057	0.185	0.847
Condition	16	10	10		0.639	0.557
Liver wt.	16	8	8	17		0.456
Gonad wt.	16	10	10	17	19	

Males

	Age	Body wt. vs age	Length vs age	Condition	Liver wt. vs wt. or L	Gonad wt. vs wt. or L
Age		0.693	0.321	0.945	0.712	0.855
Body wt. vs age	13		0.623	0.542	0.948	0.603
Length vs age	7	7		0.434	0.667	0.623
Condition	15	13	7		0.583	0.741
Liver wt. vs wt. or L	11	9	5	11		0.712
Gonad wt. vs wt. or L	15	13	7	15	11	

NOTE: Number of studies given below diagonal

Table 3. Coefficents of variation (CV) for reference fish GSI

Species	Sex	No. sites	CV of GSI (%)		
			Min.	Max.	Mean
White sucker	Female	25	6	80	28
	Male	20	11	78	29
Longnose sucker	Female	8	15	43	26
	Male	11	17	56	31
Walleye	Female	2	22	83	53
	Male	4	15	50	27
Yellow Perch	Female	3	29	60	41
	Male	4	15	53	34
Winter Flounder*	Female	4	29	68	50
	Male	5	22	67	48
Tomcod	Female	2	30	67	49
	Male	2	28	62	45

NOTE: summary includes studies with n>=10

* - GSI were mostly <1%

Table 4. Power of fish laboratory toxicity tests (from Suter et al., 1987)

Endpoint	No. tests	% reduction at MATC			Required variance for AFS (1)	
		Mean	Min.	Max.	CV (%)	antilog RMSE
Parental survival	13	20	<=0.1	61	40	1.56
Hatch	16	12	<=0.1	69	24	1.29
Fecundity	27	42	<=0.1	87	84	2.97
Larval survival	40	19	<=0.1	71	38	1.52
Weight	52	20	<=0.1	44	40	1.56
Egg weight	24	35	3	71	70	2.37
Lowest observed effect	77	28	<=0.1	84	56	1.93

NOTES: MATC = Maximum Acceptable Toxicant Concentration; =GM (LOEC, NOEC)

antilog RMSE = antilog square root Mean Square Error (MSE) for log-transformed values

CV = Coefficient of Variation

(1) Variances required for an AFS with n=22 fish per area to provide the same power as the mean or average laboratory test

Table 5. Summary of natural spatial differences observed for AFS variables

Site	Species (1)	Sex	% diff. between areas					
			Age	Growth		Condition	Liver wt. @Wt or L	Gonad wt. @Wt or L
				Wt	L			
SLPC, Alberta	WS	F	6-28	3-13	1-2	<1-6	4-7	5-19
		M	13	5	1	<1	4	9
	LNS	F	2-31	4-24	1-4	1-11	2-58	--
		M	18-33	9-26	2-9	1-5	<1-22	<1-18
James River Marathon,	LNS	F	19	14	5	<1	4	18
Ont.		M	18	14	3	6	--	18
Avenor, Dalhousie, NB	RC	F	--	--	--	5	15	--
		M	--	--	--	--	5	77
	WF	F	16	42	15	--	--	<1
		M	4	16	<1	18	8	23

NOTE: "--" indicates that differences were not reported or not calculated (usually because regression slopes differed among areas)

NA = Not Applicable

(1) LNS = longnose sucker; RC = rock crab; WF = winter flounder; WS = white sucker

Table 6. SLPC multi-year AFS - tests of spatial-temporal variance (Y{BA} x L)
(see Section 2.3.2 for details)

Species	Sex	No. tests			
		Total	p<=0.05	0.05<p<=0.20	p>0.20
White sucker	Female	8	0	4	4
	Male	6	0	0	6
	Both	14	0	4	10
Longnose sucker	Female	7	3	2	2
	Male	6	3	0	3
	Both	13	6	2	5
Both	Both	27	6	6	15

Table 7. SLPC multi-year AFS - temporal changes in spatial differences.
(see Section 2.3.2 for details)

Species	Sex	Y	X	Year	% diff.
White sucker	Female	Ovary wt.	Age, SL	1989	-5%
				1990	-19%
				1992	-1%
				1994	7%
				Post vs Pre	15%
	Male	Testes wt.	SL	1990	-9%
				1992	6%
				1994	12%
				Post vs Pre	18%
Longnose sucker	Female	Fecundity	Age, SL	1989	7%
				1990	-7%
				1991	17%
				1992	11%
				1994	7%
				Post vs Pre	12%
	Male	Testes wt.	SL	1989	0%
				1990	18%
				1991	-4%
				1992	-2%
				1994	2%
				Post vs Pre	-10%

NOTES: Difference (diff.) for white suckers is Exposed versus Reference area; difference for longnose suckers is between two Exposed areas. Post vs Pre compares average post-operational (1991-94) difference with average pre-operational (1989-90) difference

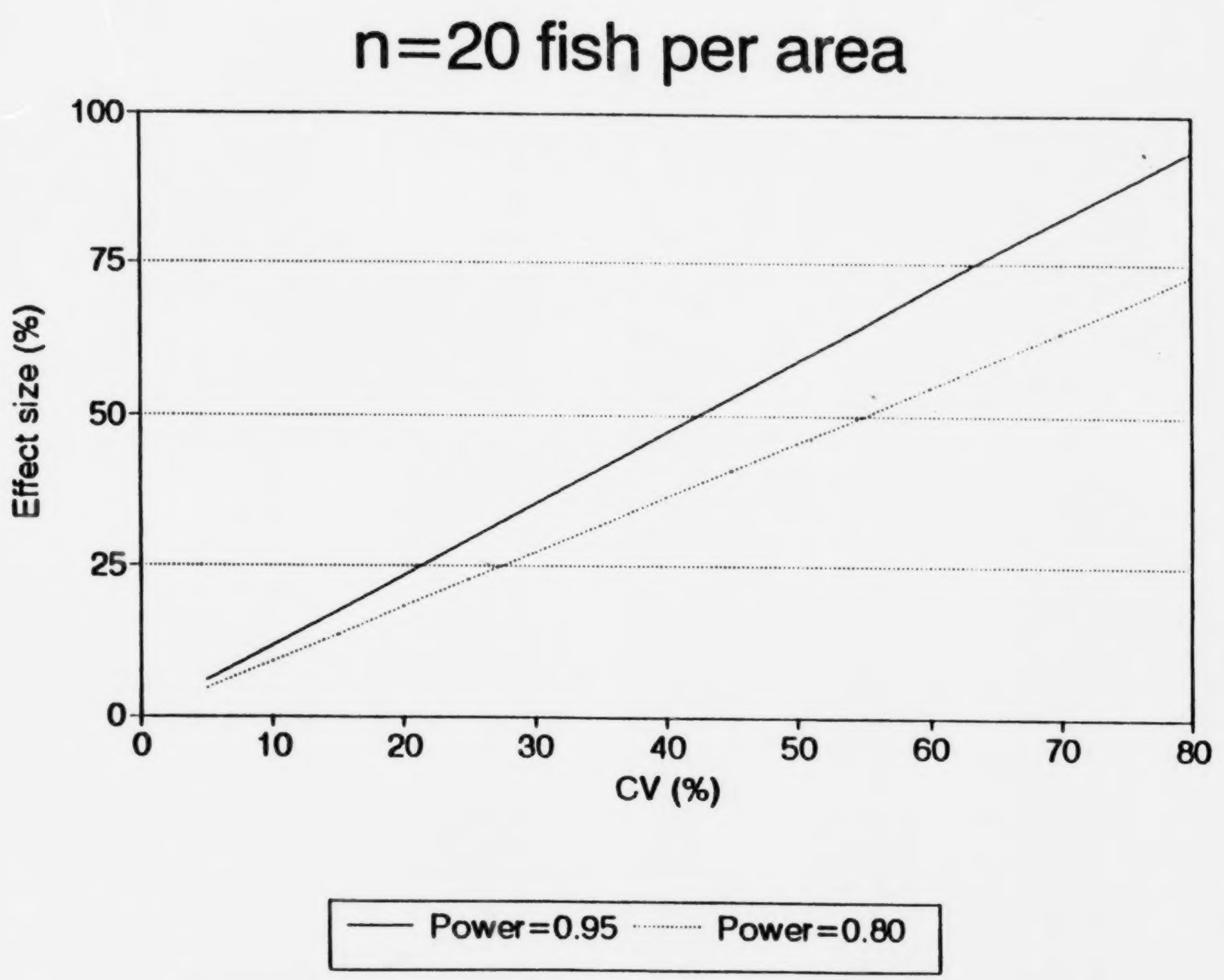

Figure 1. Effect sizes which can be detected given minimum sample size requirements from Cycle One ($n=20$ fish per area)

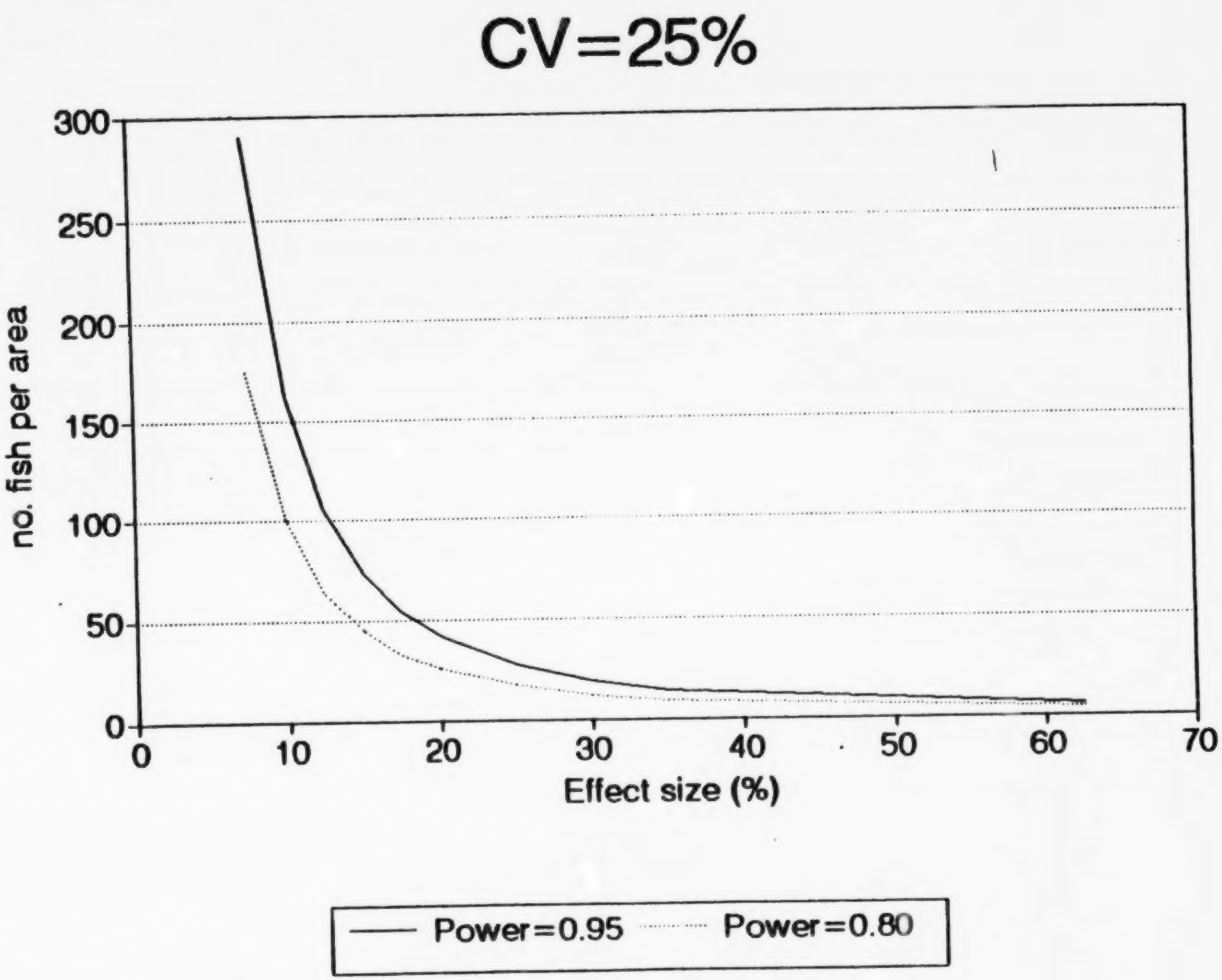

Figure 3. Sample sizes required to detect various ES, given CV=25%

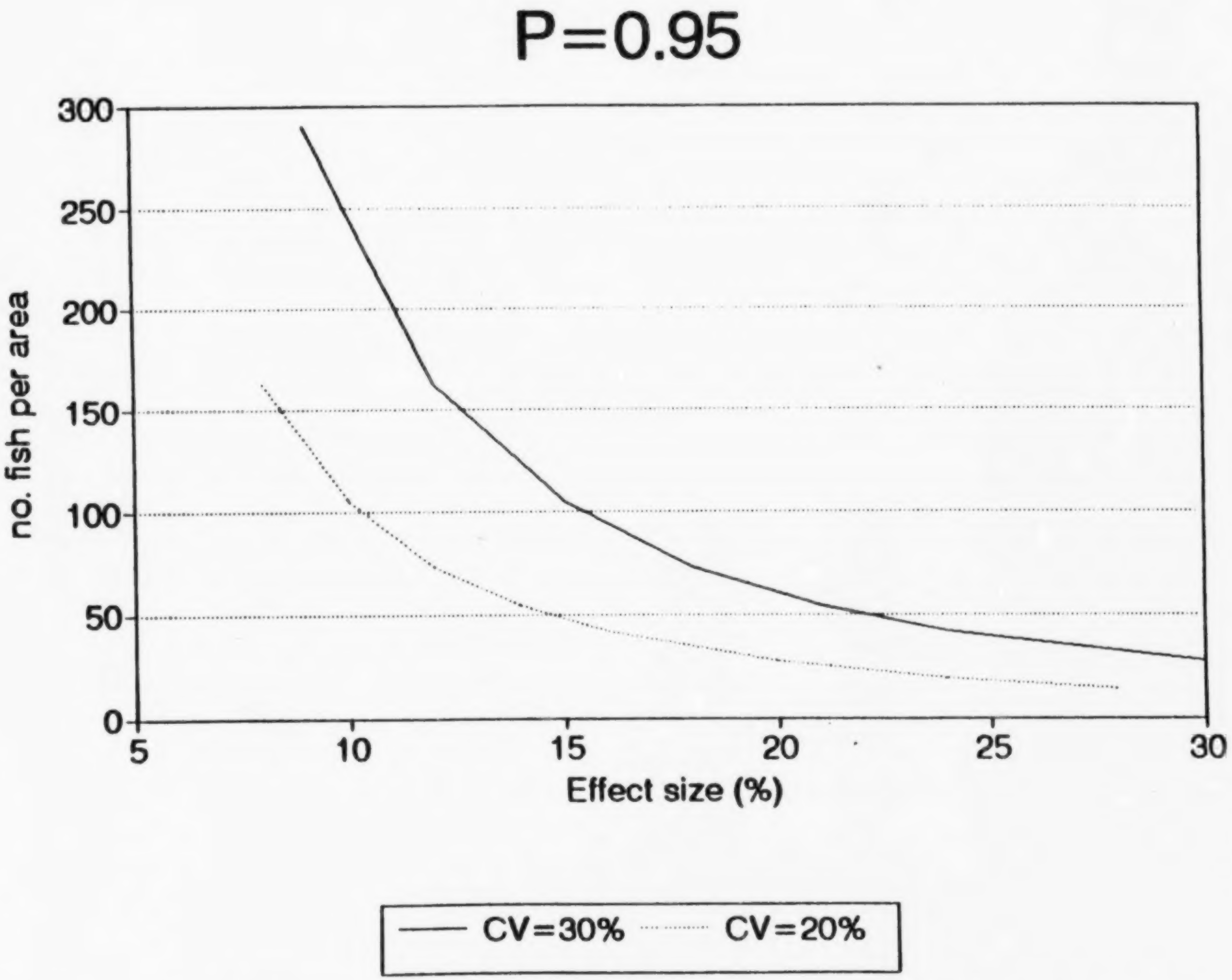

Figure 4. Sample sizes required to detect various ES, given *P*=0.95, for CV of 30% vs 20%

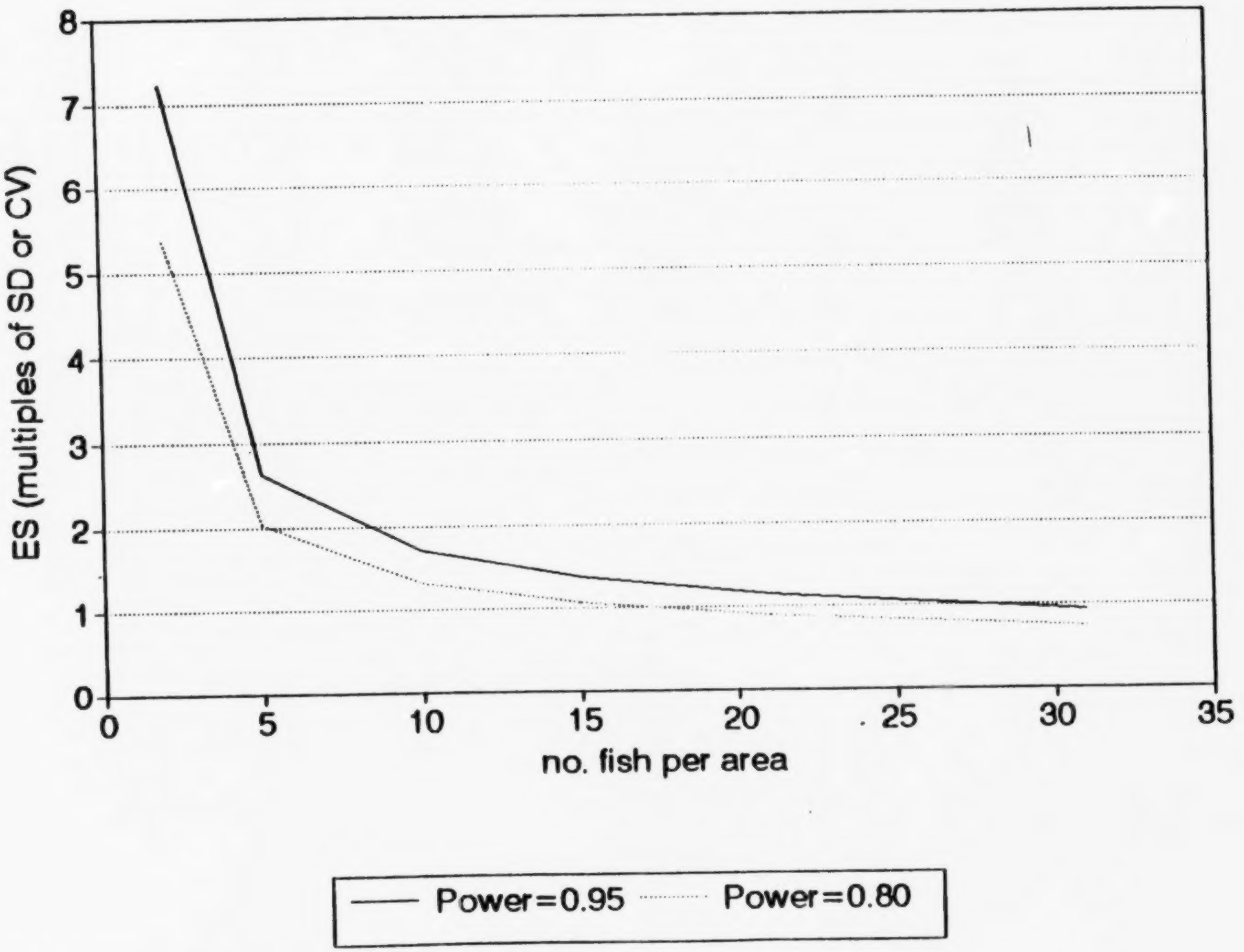

Figure 5. **ES which can be detected with small sample sizes (*n*<30)**

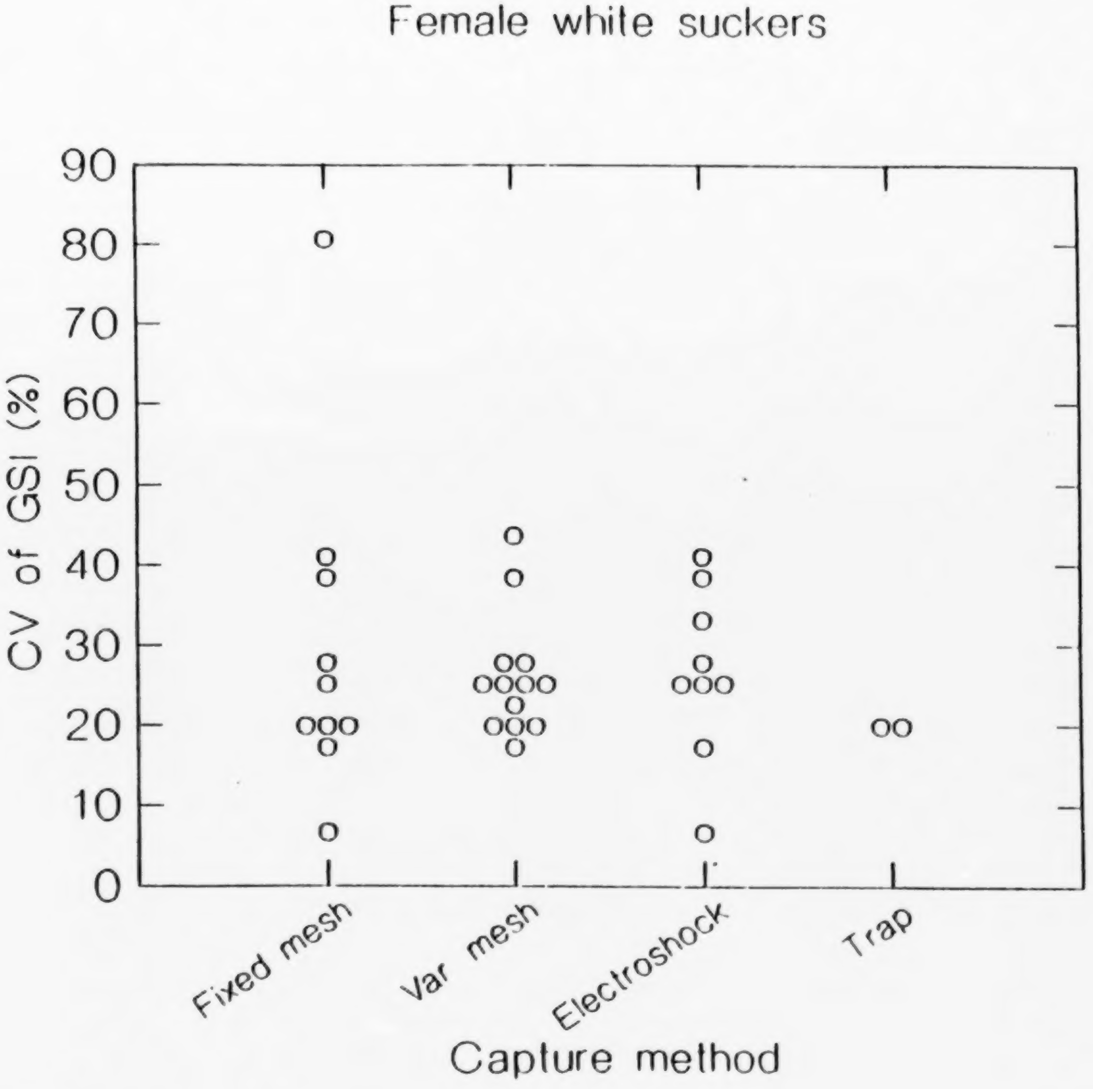

Figure 6. CV of GSI for female white suckers caught using various methods

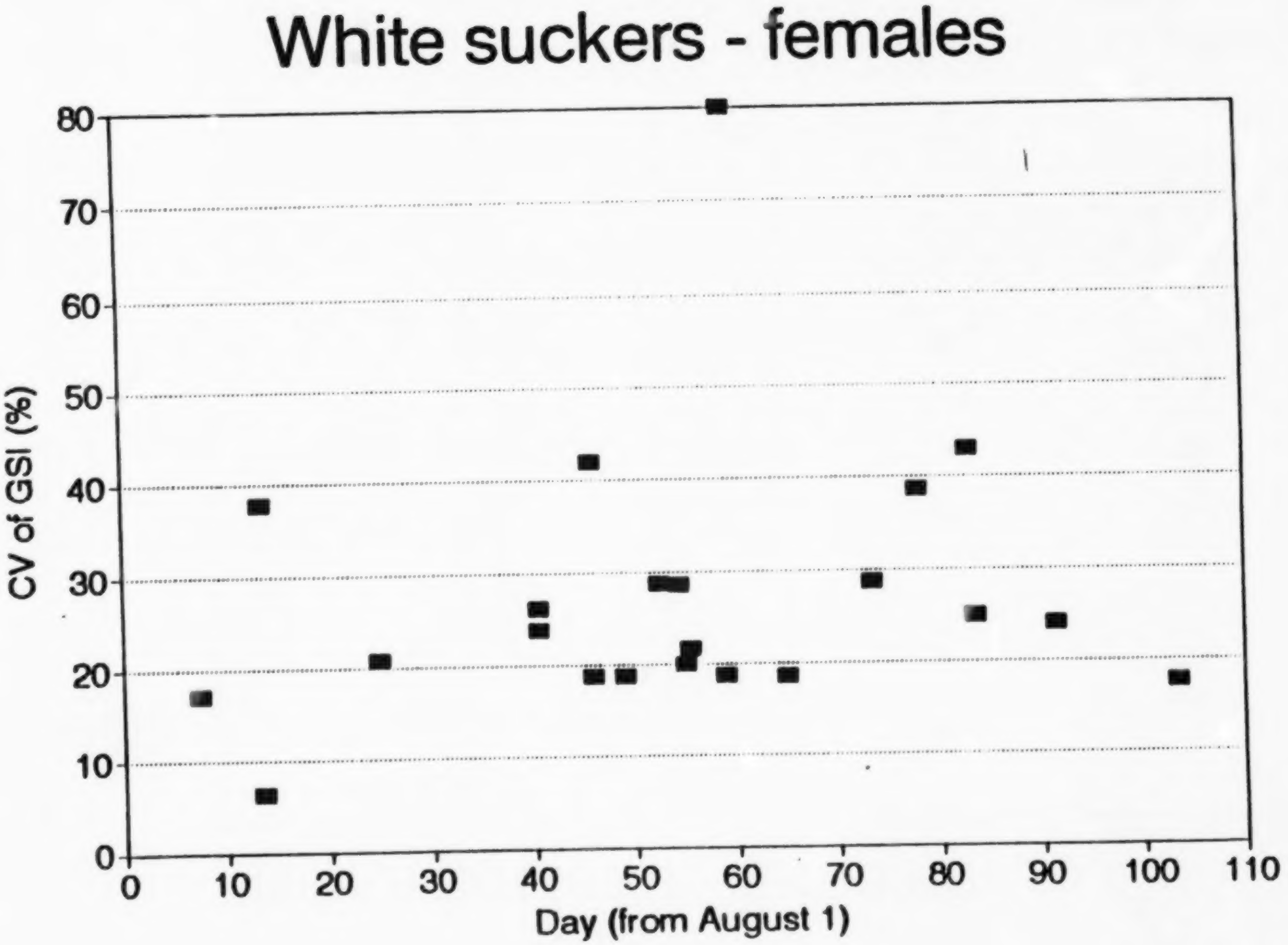

Figure 7. Relationship between CV of female white sucker GSI and mid-point of the sampling interval

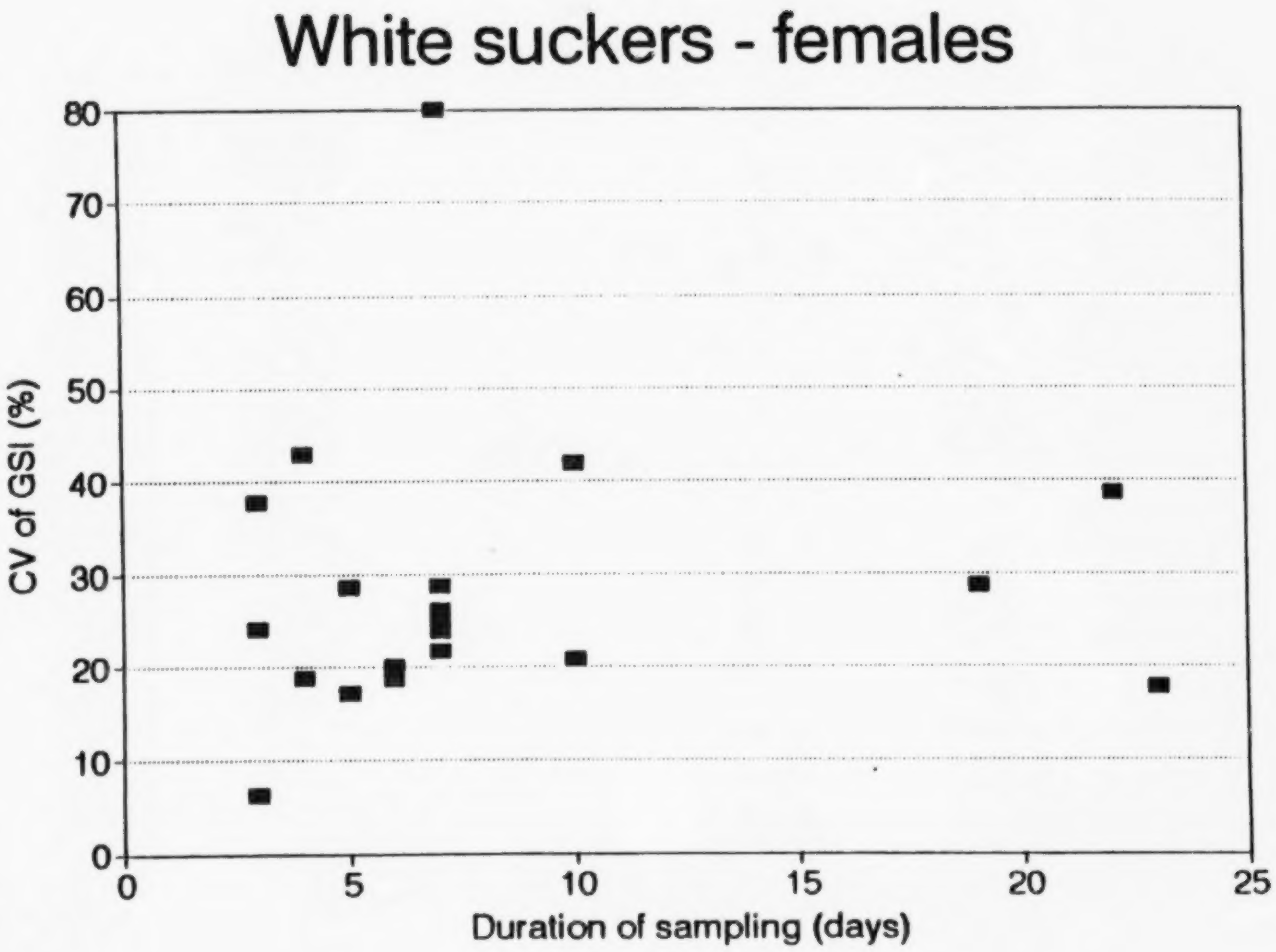

Figure 8. Relationship between CV of female white sucker GSI and duration of sampling

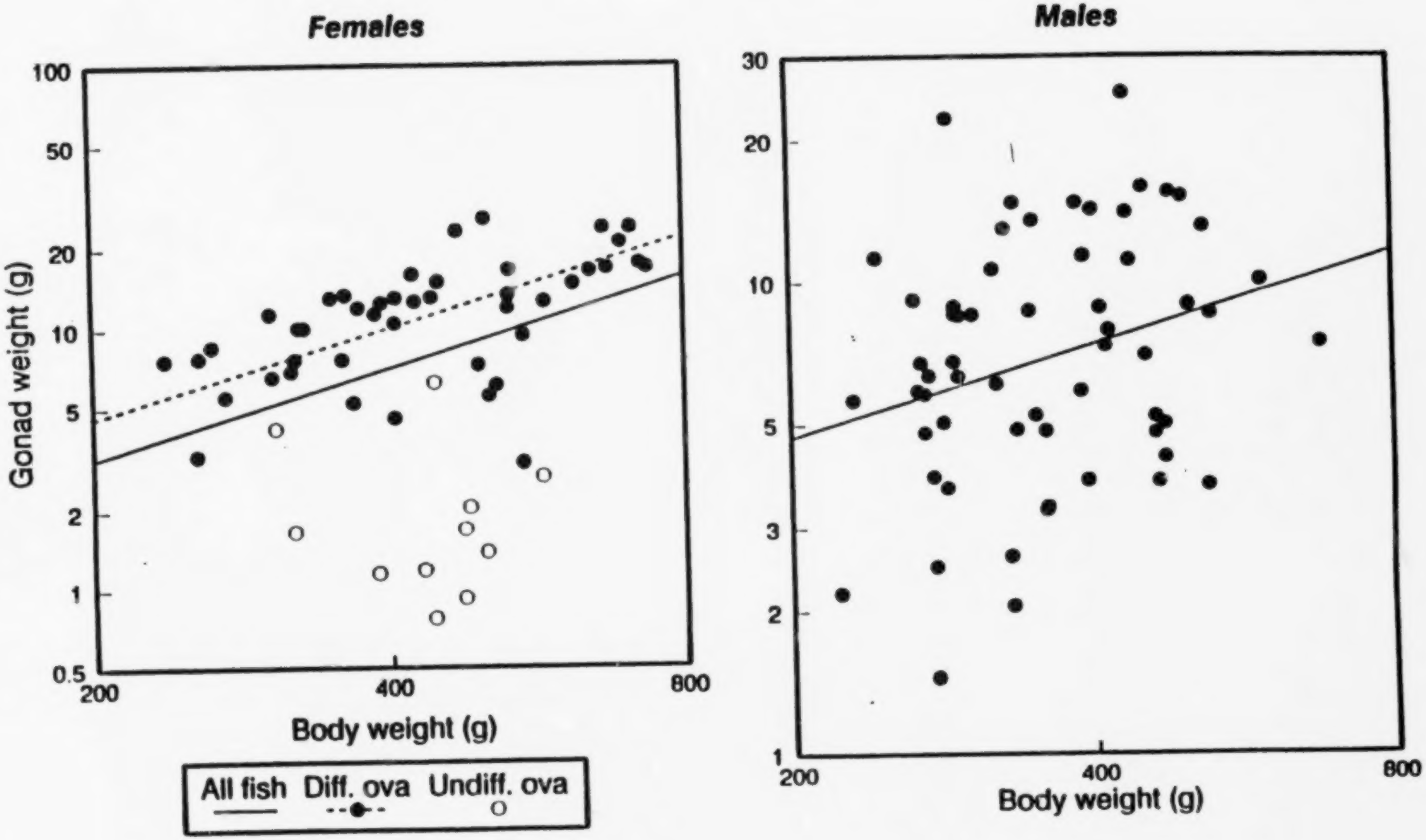

Figure 9. Gonad weight versus body weight for mountain whitefish from the Peace River, B.C.

APPENDIX II

ALTERNATIVE STRATEGIES

The following is taken from:

Courtenay, S.C., W.R. Parker, and G.P. Rawn. 1997. A workshop to assess alternatives to the fish survey component of the environmental effects monitoring program for Canadian pulp and paper mills. Can. Tech. Rep. Fish. Aquat. Sci. XXXX: vii + 101 p.

Abstract

On 17-18 February 1997 approximately 30 participants from Canada and USA gathered in Dartmouth, Nova Scotia to discuss alternatives to the Adult Fish Survey (AFS) component of Canada's Environmental Effects Monitoring (EEM) program for liquid effluents from pulp and paper mills. The workshop was a function of the national AFS Expert Working Group (EWG) which was tasked with reviewing fish surveys carried out during the first cycle of the pulp mill EEM (1993-1996) and recommending changes for the second cycle. During the first cycle of the pulp mill EEM, the AFS performed reasonably well in the freshwater environment for which it was designed, but poorly in the marine and estuarine environments. The objective of the workshop was to assist the AFS EWG in making recommendations for the second cycle and to identify promising biomonitoring approaches which require further development. Presentations were given on experiences and difficulties with the AFS in Cycle 1 across the country and on potential alternatives to be considered for Cycle 2 including: use of alternate sentinel species including macroinvertebrates and forage fish; use of alternate endpoints including health indices, scope-for-growth, behavioural and life history indices; cageing of bivalves and fin-fish; and mesocosms. In light of the principles for conducting EEM studies and the comments made by the workshop participants, the alternative methods identified as being acceptable for Cycle 2 are, in order of preference, surveys of wild forage fish (i.e., small-bodied, sedentary fish), wild bivalves, juvenile fish, caged bivalves, and mesocosm studies with fish. The workshop participants also concluded that there was a need for an increased level of research and development aimed at the provision of better monitoring tools for EEM.

As an activity of the Fish Survey Expert Working Group, a national workshop was held in Dartmouth, N.S. on 17-18 February 1997 to consider alternatives to the Adult Fish Survey. The workshop was organised and hosted jointly by Environment Canada (Roy Parker, Environmental Protection Branch, Atlantic Region) and DFO (Gary Rawn, Environmental Science Branch, Headquarters; Simon Courtenay, Marine Environmental Science Division, Maritime Region). The objectives of the workshop were to:

1. review difficulties with Adult Fish Surveys in Cycle One;
2. explore alternative approaches;
3. identify alternative approaches ready for use in Cycle Two; and,
4. identify alternatives that are not ready for Cycle Two but that merit further research and development in order that they be available for Cycle Three and beyond.

Abstracts of the 15 invited presentations and accounts of discussions are presented in this report. The workshop program and list of attendees are presented in Appendices C and D respectively. The workshop began with reviews of Adult Fish Surveys in Cycle One in the Atlantic Region, Pacific Region, and Central Canada (abstracts 1-3) and progressed to consideration of bivalves and crustaceans as sentinel species (4, Appendix E), potential applications of caged bivalves (5,6), mesocosms (7) and scope for growth (8). The first day concluded with a review of the activities of the Fish Survey Expert Working Group (9) and a discussion of the problems and potential solutions presented during the day (Appendix A). The second day began with three presentations on Health Assessment Indices (HAI) (abstracts 10-12) followed by a consideration of cage studies, behavioural indices and life cycle studies with finfish (13, Appendix F) and two presentations on the use of small, sedentary fish as sentinels (13-14). The workshop concluded with a discussion of the day's presentations and which alternatives presented during the workshop were ready for application in Cycle Two and which should be recommended for further research (Appendix B).

Based on the presentations and discussions summarized above, we have attempted to evaluate the various alternatives in light of the principles for conducting EEM studies (as published in EC/DFO 1992) and the comments made by the workshop participants. Each alternative was rated as being either: 1) Ready to be used in Cycle 2; 2) Applicable for EEM but the methods require additional work and confirmation; or 3) Not applicable to pulp and paper EEM. A summary of the results of this evaluation are summarized in the following Table. More detailed evaluations are provided in Appendix G.

Table 1. - Evaluation of the applicability of alternative methods for pulp and paper EEM studies.

Alternative Method or Approach	Ready for Cycle 2	More Work Required	Not Applicable to EEM
Wild bivalve survey	•		
Caged bivalve studies	•		
Habitrap		•	
Bivalve Scope for Growth		•	
Mesocosm with fish	•		
Health Assessment Index		•	
Invertebrate Pathology		•	
Caged Finfish			•
Forage fish - marine	•		
Forage fish - freshwater	•		
Juvenile fish survey	•		
Salmon life cycle study		•	

In general, consensus was that if the fish survey in Cycle 1 was successful, or could be made successful by better capture techniques, timing, sentinel selection, reference site choice, and there are no serious confounds with other effluent discharges or historical damage (e.g., substrate alteration by fibre mats, wood chips), then a similar, standard fish survey should be followed in Cycle 2. To ensure exposure to effluent in the nearfield, species should be sedentary (e.g., the mummichog) or there must be a working tracer. Given the poor record of tracers in Cycle 1 and their associated expense and logistical problems, it seems preferable to work with a sedentary species if such are available. Where possible, animals exposed consistently to $\geq$ 1% effluent should be sampled but where this is not practical, animals near the discharge point should be sampled. However, effluent discharged into currents that are rapid and/or variable in speed and direction may have most effect some distance from the end of the pipe. This is particularly true in marine and estuarine receiving environments. Mills carrying out fish surveys in such environments should seek advice from a qualified hydrologist or physical oceanographer before establishing impact and reference sites. Where sedentary finfish species are not available, wild bivalves are suitable sentinel species. In general, crabs and lobsters cannot be aged (Appendix E) and should be avoided. Every effort should be made to sample two sentinel species but if only one appropriate sentinel species can be found, the survey might proceed with just the one.

Some areas are not inhabited by adult fin-fish or macroinvertebrates but are nursery areas for their juveniles. In such cases, it would seem to make sense to replace the standard fish survey by a survey of juvenile fish. Relevant measures would include: assessment of percent survival, incidence of developmental problems associated with exposure to pulp mill effluent (e.g., vertebral fusions and compressions, lordosis, scoliosis, fin erosion), and growth in juveniles exposed to pulp mill effluent, compared to juveniles in otherwise comparable reference sites. In some areas, such a survey might be precluded by insufficient knowledge of distributions to establish reference and exposure sites, high temporal and geographic variability, or inability to determine age and therefore growth.

If a fish survey cannot be carried out, either because the receiving environment cannot be sampled or because insufficient fin-fish or shellfish are found there, the preferred alternative would be a caged bivalve exposure. In many cases, this approach will be able to circumvent problems with confounding discharges and historical damage such as substrate alteration. In situations where a caged bivalve exposure cannot be carried out because the receiving environment is inappropriate (e.g., strong currents, fluctuating salinities, confounding effluents, historical damage (e.g., substrate alteration)), a mesocosm exposure is a suitable alternative. Because neither caged bivalves nor finfish in mesocosms have been used previously in EEMs for Canadian pulp and paper mills, demonstration projects are scheduled to be carried out during 1997.

Recommendations

1. Based on the presentations made at the workshop, the discussion of the various alternatives by workshop attendees and the evaluation of the alternatives provided in this report; it is recommended that the Fish Survey Expert Working Group and the Technical Management Committee (TMC) for the pulp and paper EEM program make provisions for the inclusion of applicable alternatives in Cycle 2. These include: surveys of small, sedentary (forage) fish and bivalves, caged bivalve studies and mesocosm studies with fish. In addition, surveys of juvenile fishes should be an acceptable alternative in receiving environments that are nurseries for fish but do not serve as habitat for adult fish.
2. Where possible, the TMC should encourage and sponsor protocol development and pilot studies to facilitate technology transfer to the consulting community. Pilot studies to demonstrate the utility of caged bivalves and mesocosms for fish should proceed immediately in order that reports are available to industry before the end of 1997.
3. The TMC should encourage and sponsor research for those alternatives that require further refinements.

EXTENDED ABSTRACTS

1. Experiences with the first cycle EEM adult fish surveys in the Atlantic Region

W. Roy Parker
Environmental Protection Branch.
Environment Canada, Dartmouth, NS

During the first EEM cycle for the pulp and paper industry in the Atlantic Region, there were nineteen mills operating and subject to the requirements of the Pulp and Paper Effluent Regulations. In total, 17 EEM studies were conducted; there were 2 joint studies. Of the 19 mills, 4 discharge to freshwater, 8 discharge to estuaries and the remaining 7 mills discharge directly to the ocean. At the time of the EEM studies, 8 mills had secondary treatment and the other 11 had primary effluent treatment only.

The EEM studies conducted at mills that discharge to freshwater used three sentinel species: white sucker (2 mills); yellow perch (2 mills) and threespine stickleback (1 mill). At mills that discharged to the marine environment, a total of 10 different sentinel species were used. The most commonly used sentinel species were the Atlantic tomcod (7 mills) and winter flounder (8 mills). Green crab and rock crab were each used at 3 mills. The remaining sentinel species; Atlantic silversides, cunner, longhorn sculpin, shorthorn sculpin, American lobster and mummichog were each used at one mill. The most common fishing techniques used were gill netting and trap netting. Other methods used to capture fish included baited long lines, minnow traps, electrofishing, otter trawling and SCUBA divers.

Only one EEM study captured the minimum required numbers of 20 males and 20 females for 2 sentinel species at both a reference and effluent exposure site. For 9 other EEM studies, the minimum numbers were captured at both sites for one sentinel species.

The following is an example of an adult fish survey (AFS) at a mill discharging to freshwater. The minimum numbers of targeted sentinel species, white sucker and yellow perch, were captured. Measurement of resin and fatty acids in the bile of a sub-sample of the sentinel fish indicated that the fish from the exposure area had been exposed to pulp mill effluent. The measurements of these fish and the analysis of the data indicated that there were significant differences between reference area fish and exposure area fish. Overall, this study met the objectives of cycle 1.

At another mill discharging to freshwater, threespine stickleback were the only non-migratory fish in the river and were used as the sentinel species. The minimum number of fish were not captured in both the exposure and reference areas. The reference and exposure areas were separated by a hydroelectric dam so a tracer was not required to confirm exposure. Measurements on the captured fish indicated that they were all of the same age class. The only significant difference between exposure and reference fish was in female length. In summary, the cycle 1 objectives were not attained for this mill.

The following is a summary of the adult fish survey for a mill discharging to marine waters. The selected sentinel species were winter flounder and rock crabs. Although the minimum numbers of both sentinel species were not captured, the results indicated that the minimum numbers were almost achieved. The use of tracers was not successful at confirming effluent exposure. The measurements made on the fish and the data analyses indicated many differences between the fish in the reference and exposure areas. Unfortunately, because the crabs could not be aged, the data were incomplete. Overall, the cycle 1 adult fish survey was partially successful.

The final example is for a mill discharging to a very dynamic estuary system. For sentinel species, the Atlantic tomcod, green crab and mummichog were selected. The fishing effort was not successful at capturing the minimum number of any of these fish in both the reference and exposure areas. Due to the limited number of specimens, the comparison between fish from the different areas were not possible. Attempts to confirm the exposure of the fish to effluent were not successful. Overall, the cycle 1 adult fish survey was not useful at this site.

A review of all of the adult fish survey results indicated that this EEM component provided useful information at two of the freshwater mills and at seven of the marine mills. At one freshwater mill and at the remaining eight marine mills, the first cycle adult fish survey was not very useful. Some of the problems experienced with the AFS during cycle 1 included not capturing enough fish, finding suitable sentinel species, using crustaceans which could not be aged, finding suitable reference sites, the confounding of results by other pollution sources and the lack of confirmation of effluent exposure.

For the next cycle of EEM studies, a continuation of an AFS type of approach would be appropriate at about half of the Atlantic Region mills. At the other half of the mills, it does not make a lot of sense from a scientific or cost effectiveness perspective to continue with an AFS approach. If a different method of assessing the health of crustaceans can be developed which does not require aging, the AFS could be continued a few more mills. It is obvious that alternate means of assessing fish community health will be required at a number of sites for the next EEM cycle.

Questions, Answers and Comments

Q. Where exposure to effluent was not confirmed, was this because no tracer was detected?

A. Tracers were sometimes detected but no difference was seen between the effluent and reference sites.

Q. Is it possible to age crustaceans and if not, why use them?

A. Ageing decapods is not generally possible though we can still compare sizes, quality etc. Perhaps we have learned during Cycle 1 that these animals are not the best sentinel species to use in Cycle 2. You can mark individual crabs and know that they have been exposed but if we don't know how they respond to exposure, we can't use them.

Q. Only one mill caught enought fish and only 3 determined exposure so how can you say that 9 were successful?

A. At least partial success was seen at 9 mills and with greater effort and better tracer methodology, we will do better in Cycle 2. Remember that Cycle 1 was exploratory.

Q. One objective of Cycle 1 was to determine required sample sizes for Cycle 2. What did you find?

A. Sample size depends on what magnitude of effect you want to detect. Twenty fish may be adequate for detecting a 50% difference in liver size but not a 20% difference for example.

2. EEM Cycle One Adult Fish Survey Experience: Pacific and Yukon Region

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Environment Canada, Pacific & Yukon Region

Before evaluating the EEM Adult Fish Survey (AFS) it is necessary to have a clear understanding of the distribution of mill effluents in the environment. Knowing the dilution and dispersion characteristics for each mill effluent is a key element in study design: it determines the exposure/dose of the effluent to the biological receptors (in this case, the fish sentinel species). Similarly, one of the prime objectives of the Cycle One AFS was the evaluation of the potential sentinel species and the confirmation of the validity of the reference areas.

The 23 pulp and paper mills that conducted an EEM program in British Columbia (Figs. 2.1 and 2.2) discharge to a wide range of receiving environments. Eastern B.C. mills discharge to clear rivers, two of which are downstream of hydro dams (Fibreco CTMP on the Peace River and Celgar kraft mill on the

Columbia River). The mill at Crestbrook discharges tertiary treated kraft effluent from a colour clarifier to the glacially clear Kootenay River. The two mills at Mackenzie are located on the Williston Reservoir which also possess unique challenges to conducting an EEM program given the 17 m draw-downs which occur. The Fraser River, which carries a high suspended solids load, directly receives the discharge of four kraft and CTMP mills in the Prince George-Quesnel area, and indirectly receives the effluent from one kraft mill on the Thompson River tributary at Kamloops.

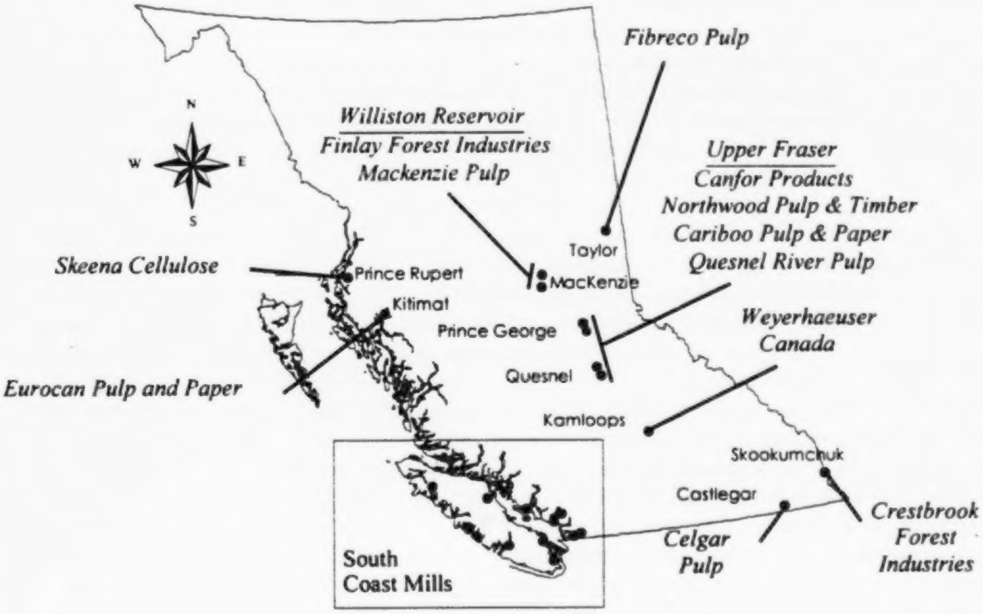
Fibreco Pulp
Williston Reservoir
Finlay Forest Industries
Mackenzie Pulp
Upper Fraser
Canfor Products
Northwood Pulp & Timber
Cariboo Pulp & Paper
Quesnel River Pulp
N
W
E
S
Taylor
Skeena Cellulose
Prince Rupert
MacKenzie
Kitimat
Prince George
Weyerhaeuser
Canada
Quesnel
Eurocan Pulp and Paper
Kamloops
Skookumchuk
Castlegar
Crestbrook
Forest
Industries
Celgar
Pulp
South
Coast Mills

Figure 2.1: Pulp and Paper Mills in North and Interior British Columbia

The marine and estuarine mills discharge to environments which range from riverine to oceanic. Two paper mills, Scott and Paperboard, located in the estuarine portion of the Fraser River at Vancouver, and the CTMP mill in the Somass estuary at Port Alberni, have shallow, and in the case of the paper mills, low-volume discharges (8,000-15,000 m^3/d). This is in marked contrast to the large-volume (85,000 to 270,000 m^3/d), deep (73-115 m) sea outfalls of the Port Mellon, Harmac, and Powell River kraft mills. The effluent from these three outfalls have been shown through dye studies to remain trapped at 20-60 m below the sea surface (Table 1). At the other mixing extreme is the mill at Elk Falls which discharges effluent at 15-40 m but this effluent is rapidly mixed due to the 6 m/s currents of Discovery Passage. Similarly, well-mixed effluent is found at the Skeena kraft mill discharging to Porpoise Harbour. The mills at Port Alice, Woodfibre, and Gold River have relatively shallow discharges (20-40 m) which allow the effluent to rise to the surface (0-10 m).

A range in length of 1% effluent dilution zones is apparent for marine and estuarine mills (Table 2.1). The largest 1% dilution zones are in estuaries where a large volume of effluent enters the surface layer and is transported down-inlet (5 - 24 km). The smallest dilution zones (<500 m) consist of a variety of low and high volume, trapped and surfacing effluents, released into relatively high energy environments. Effluent mixing may be less complicated for freshwater mills, but should not be assumed to occur instantaneously. The 1% dilution zones range from 50 m to 7 km under most conditions, but may reach 85 km below Crestbrook and 500 km below the Upper Fraser mills during periods of high effluent discharge and very low river flows.

For the AFS, mills were required to sample two sentinel species, and were given a minimum sample size target of 20 adult males and 20 adult females for each species. Sampling areas included at least one reference area and an effluent-exposed area, many mills also included a far-field fish sampling area. There was a further requirement to confirm that samples were representative of these areas by looking for an appropriate mill tracer. To this end, ambient water was sampled at 18 mills and fish tissues were sampled at nine mills. In general, water column tracers were more successful than tissue tracers, and tracers were more successful in freshwater environments than in marine environments. At one freshwater mill (Crestbrook) dioxins and furans indicated that reference mountain whitefish had previously been exposed to effluents and were thus not a suitable sentinel species, in contrast to the dioxin monitoring at Celgar which confirmed the integrity of mountain whitefish and large scale suckers species for that mill. (Table 2.2). Most marine mills located exposed and reference sites for AFS crab sampling based on dioxin and furan data from crabs collected for their dioxin monitoring programs. These data are not considered in this table. No marine mills were able to confirm exposure using chemicals in fish and only four of nine marine and estuarine mills confirmed exposure using a water column tracer. A complete analysis of tissue tracers from mills in Canada is reported elsewhere.

Table 2.3 presents an overview of capture and analysis success for marine/estuarine and freshwater sentinel species. Only one marine and four freshwater mills caught the requisite number samples of two

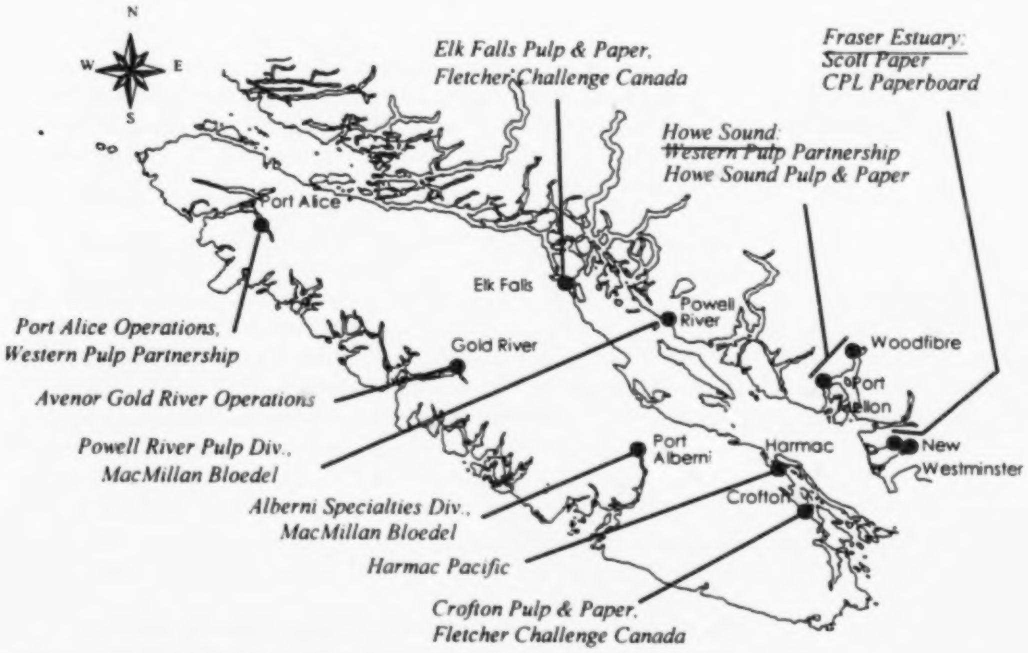
N
W
E
S
Elk Falls Pulp & Paper,
Fletcher Challenge Canada
Fraser Estuary:
~~Scott Paper~~
CPL Paperboard
Howe Sound:
~~Western Pulp~~ Partnership
Howe Sound Pulp & Paper
Port Alice
Elk Falls
Powell River
Woodfibre
Port Mellon
Gold River
Port Alberni
Harmac
New Westminster
Crofton
Port Alice Operations,
Western Pulp Partnership
Avenor Gold River Operations
Powell River Pulp Div.,
MacMillan Bloedel
Alberni Specialties Div.,
MacMillan Bloedel
Harmac Pacific
Crofton Pulp & Paper,
Fletcher Challenge Canada

Figure 2.2: Pulp Mills on the South Coast of British Columbia

sentinel species at both exposed and reference sites; six mills included a far-field location in addition to the near-field and reference locations. The majority of mills were able to catch at least one sentinel species, though five of the eleven marine mills used a bivalve species to meet this requirement. Only one marine mill was unsuccessful at obtaining the required number of at least one sentinel species (due to the high current speeds in Discovery Passage and the segregation of male and female crabs in the environment). One Fraser River estuary mill caught two species in adequate numbers, but they were predominately juveniles. These findings were reflected at recent regional workshops (Colodey 1996) and underscore the need for alternative approaches in marine and estuarine environments. The use of wild or caged mussels or oysters may be useful ways to assess the impact of effluents. Caged shellfish offer the opportunity to assess growth and condition changes after periods of known exposure and can provide adequate replication for statistical purposes. Mesocosms may also allow for the controlled exposed of selected species as a substitute for locations which are confounded by multiple discharges.

Table 2.4 compares capture success of finfish and invertebrate species in relation to the number of times they were proposed in study designs. In general, freshwater fish were more often used as proposed in the study design and had a higher rate of successful capture compared to marine fin fish. Large-scale sucker (LSS) was the most common freshwater species (five mills) and blue mussels (three sites), English sole (two sites) and prickly sculpin (two sites) top the list for marine and estuarine mills.

When the performance of various species from marine and estuarine environments are considered (Table 17, App. III) a number of problems are evident above and beyond the numbers of fish caught: juveniles predominate (starry flounder, prickly sculpin), sexes not equally caught (Dungeness crab, widow rockfish), old fish or difficult to age (Widow rockfish, threespine stickleback), and batch spawners (threespine stickleback). Species which were plentiful in the marine environment at some locations include blue mussels, Pacific oysters, and Dungeness crab. Unfortunately crabs cannot be reliably aged and are subject to commercial and recreational harvesting, thus limiting their usefulness as a sentinel organism. Other shellfish (oysters or mussels) had reduced condition factors in near-field locations compared to reference sites (Crofton, Powell River, Port Mellon, Woodfibre), although in the latter three cases the reference area was not suitable. Fishing success for freshwater mills was higher and sex distribution was more evenly distributed than for the marine and estuarine mills (Table 17, App. III).

Few conclusions regarding differences between exposed fish in relation to reference sites are possible given the exploratory nature of cycle one EEM sampling. Further examination of changes in fish will be done on a species and sex specific basis to determine if changes to, e.g., LSS are consistent between locations, although a cursory examination of the data would not seem to confirm this.

CONCLUSIONS

1. It is essential to have a good knowledge of effluent distribution in the environment prior to selecting sentinel species, given the problems associated with the use of tracer compounds.
2. Other discharges and confounding habitat factors need to be considered and underscore the usefulness of coordinated monitoring through a basin approach where other dischargers also monitor the sublethal and environment effects of their effluents. Do not assume because you are "downstream" of a pulp mill that it is the only factor influencing what you are sampling.
3. There is a need for alternatives to the Fish Survey approach as outlined for Cycle 1 EEM: mesocosms for highly confounded areas; wild and/or caged mussels for areas where finfish cannot be used due to exposure or catch difficulties.
4. There may be instances where a Fish Survey is not practical due to either lack of exposure, confounded exposures, or lack of appropriate sentinel species.

REFERENCES

Colodey, A.G. 1996. Environmental Effects Monitoring Workshop Summary. Unpublished meeting notes. May 27-30, 1996. Institute of Ocean Sciences, Sidney, B.C.

Questions. Answers and Comments
Q. How can you quantify exposure to effluent in wild fish?
A. This cannot be certain without cageing.
Q. Did any studies do tagging?
A. Mark Spafford did some.
C. Juvenile fishes should not be written off.
C. To help address questions about exposure, some mills used multiple reference sites.
C. We should remember that the mandate of Cycle 1 was to determine whether existing regulations are adequate to protect the environment. This is much more difficult than simply determining whether effluent has the potential for harming fish. With this in mind, we shouldn't be too hard on ourselves as we review the often imperfect results of Cycle 1.

Table 2.1: Characteristics of effluent discharge - B.C. marine and estuarine mills

MILL	**Receiving Environment**	**Effluent Volume (x1000 m3/d)**	**Diffuser Depth (m)**	**Trapping Depth (m)**	**Length 1% Zone max. (km)**
Port Alberni	Somass R. Estuary	87	surface	0 - 7	24
Port Alice	Neuroutsos Inlet	136	20 - 40	0-6	10
Eurocan	Kitimat R. Estuary	63	river	none	5
Gold River	Muchulat Inlet	85	24	0-10	4
Powell River	Malaspina Strait	270	57 - 73	15-50	3
Woodfibre	Upper Howe Sound	66	22.5	2 - 8	3
Port Mellon	Lower Howe Sound	85	30 - 115	15-50	2
Crofton	Stuart Channel	154	18 & 28	0 (8-15)	0.6
Skeena	Porpoise Harbour	123	20	none	0.5
Paperboard	Fraser R. Estuary	8	river	none	0.3
Elk Falls	Discovery Passage	168	15 - 40	none	0.25
Harmac	Northumberland Ch.	143	105	20 - 60	0.25
Scott Paper	Fraser R. Estuary	15	river	none	0.1

Table 2.2: Confirmation of fish exposure to effluent at 23 B.C. pulp mills

Mill	Tissue Tracer Used[1]	Species and Tissue Used	Exposure Confirmed	Water Column Tracer Used[3]	Exposure Confirmed
MARINE AND ESTUARINE MILLS					
ALBERNI	None	na	na	Colour	Yes
CROFTON	None	na	na	Colour, CP, R/FA, T/L	No
ELK FALLS	None	na	na	Colour, CP, R/FA, T/L	No
EUROCAN	R/FA	Flounder, sculpins liver, gall bladder	No	Sodium	Yes
GOLD RIVER	Dioxin[2]	Widow rockfish muscle	No	None	na
HARMAC	CPs	English sole liver	No	None	na
PAPERBOARD	R/FA	Staghorn sculpin liver	No	None	na
PORT ALICE	None	na	na	SSL, CP, R/FA	Yes
PORT MELLON	None	na	na	Colour, CP, R/FA	No
POWELL	None	na	na	Colour, CP, R/FA	No
SCOTT	None	na	na	None	na
SKEENA	None	na	na	Colour, CP, R/FA	Yes
WOODFIBRE	None	na	na	Colour, CP, R/FA	No
13 MILLS	**9**		**0**	**9**	**4**
FRESHWATER MILLS:					
CANFOR	None	na	na	Na, CP, R/FA	Yes
CARIBOO	None	na	na	Na, CP, R/FA	Yes
CELGAR	Dioxin/furan	LWF, LSS liver, muscle	Yes	Na, CP, R/FA	Yes
CRESTBROOK	Dioxin/furan	LWF, LSS liver, muscle	Yes	Na, CP, R/FA	Yes
FIBRECO	R/FA	MWF, LNS bile	Males	None	na
FINLAY	CP, R/FA	LWF, LSS liver	No	R/FA	Yes
MACKENZIE	CP, R/FA	LWF, LSS liver	No	6 Cl-Vanillin	Yes
NORTHWOOD	None	na	na	Na, CP, R/FA	Yes
QUESNEL	None	na	na	Na, CP, R/FA	Yes
WEYERHAEUSER	None	na	na	Na, CP, R/FA	Yes
10 MILLS	**5**		**2.5**	**9**	**9**
TOTAL 23 MILLS	**9**		**2.5**	**18**	**13**

NOTES:

[1] Dioxin and furan also used at marine mills - See Dioxin and Furan section

[2] In addition to Dioxin and Furan Monitoring Program

[3] CP - Chorophenols; R/FA - Resin & Fatty Acids; T/L- Tannins & Lignins; Na - Sodium

Table 2.3: Overview of capture and analysis success from 23 B.C. pulp mills

Meets requirements for two sentinel species:

Marine/Estuarine:	**1**	Alberni[1]
Freshwater:	**4**	Canfor/Northwood, Fibreco, Weyerhaeuser[2]

[1] 18 male English sole captured at reference site

[2] 17 male Peamouth chub captured at exposed site

Meets requirements for one species at exposed site:

Marine/Estuarine:	**10**	Eurocan, Gold River, Harmac, Port Alice, Paperboard,Crofton[3], Port Mellon[3], Powell River[3], Skeena[3], Woodfibre[3]
Freshwater:	**6**	Cariboo/Quesnel, Celgar, Crestbrook, Finlay/ Mackenzie[4]

[3] 30 or more unsexed mussels or oysters captured at exposed site

[4] 19 male Lake whitefish captured at exposed site

Requirements not satisfied for either species:

Marine/Estuarine:	**2**	Elk Falls, Scott
Freshwater:	**0**	none

Table 2.4: Capture success for fish used in B.C. EEM Cycle 1

Finfish:		***Proposed***	**Captured**	**Successful**
Marine/Estuarine:				
English sole	*Parophrys vetulus*	9	3	1
Prickly sculpin	*Cottus asper*	3	3	2
Starry flounder	*Platichthys stellatus*	3	3	0
Staghorn sculpin	*Leptocottus armatus*	2	2	0
Threespine stickleback	*Gasterosteus aculeatus*	1	1	1
Quillback rockfish	*Sebastes maliger*	0	1	0
Rock sole	*Lepidopsetta bilineata*	0	1	0
Slender sole	*Lyopsetta exilis*	0	1	0
Widow rockfish	*Sebastes entomelas*	0	1	0
Pacific hake	*Merluccius productus*	1	0	0
Shiner perch	*Cymatogaster aggregata*	1	0	0
Freshwater:				
Largescale sucker	*Catostomus macrocheilus*	10	8	5
Peamouth chub	*Mylocheilus caurinus*	7	5	2
Longnose sucker	*Catostomus catostomus*	3	4	1
Mountain whitefish	*Prosopium williamsoni*	3	3	2
Lake whitefish	*Coregonus clupeaformis*	2	2	0
Northern squawfish	*Ptychocheilus oregonensis*	1	0	0

Invertebrates:

Marine:				
Dungeness crab	*Cancer magister*	5	7	4 x 0.5
Blue mussels	*Mytilus edulis*	1	3	3
Pacific oyster	*Crassostrea gigas*	2	2	2
Red rock crab	*Cancer productus*	0	1	0
Two spot prawn	*Lampetra ayresi*	0	1	0

NOTES:

There were **10** mills discharging to marine waters, **3** discharging to estuaries, and **10** discharging to freshwater.

Species **proposed** in the Design Study were not always **captured** and were **successful** only if 20 male and 20 female were collected from both exposed and reference areas.

3. ADULT FISH SURVEY IN ONTARIO REGION: EXPERIENCES FROM CYCLE 1 ENVIRONMENTAL EFFECTS MONITORING.

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Twenty-seven pulp and paper mills in Ontario conducted an adult fish survey during the first cycle of the regulated environmental effects monitoring program. The mills discharge into a variety of unique freshwater receiving environments ranging from small headwater streams and human-made historic canals to large rivers and lakes. One mill discharges its effluent to an enclosed waterlot.

Adult fish were captured mostly in autumn, using a number of techniques, including gill nets, trap nets, seine nets and electroshocking equipment Frequently, the targeted fish species were not present in sufficient numbers during field work, and alternate species were collected and measured instead. In most cases, the level of fishing effort was 7 days or less. The incidental sacrifice of non-target fish was high at some sites and included large schools of migrating lake whitefish and lake trout and also sportfish such as muskie and northern pike. Environment Canada staff conducted field audits at twenty-one mills to verify that collection sites and procedures were in accordance with the authorized study design.

Interpretive reports indicate that the mills and their consulting laboratories had reasonably good success with completing the required fish measurements, including age, length, body weight, liver weight, and external/internal physical condition. Somewhat problematic, but only in a few cases, was the requirement to measure gonad weight and egg size. The requirement to analyze for an effluent tracer in fish tissue also presented problems at some sites.

Data analysis and interpretation revealed that differences between exposed and reference fish from site to site varied considerably. These differences have been difficult to link to any particular influence given the complexity of many Ontario mill receiving environments, including the presence of other effluents and in-water structures; a limited one percent effluent plume; fish mobility; and habitat variability between the reference and exposure zones.

The adult fish survey experience in Ontario has lead to a number of recommendations for the Fish Survey Expert Working Group to consider during their evaluation of cycle 1 results, including:

1. examine the adult fish survey data from Cycle 1, especially in light of confounding factors;
2. consider passive alternatives to the adult fish survey (e.g., community assessments), especially for receiving environments which support sensitive or limited fisheries; and,
3. provide a decision tree to aid in Cycle 2 fish survey negotiations between government/mills, especially for those sites where the adult fish survey did not seem appropriate during Cycle 1.

Questions, Answers and Comments

Q. What is meant by community assessments?

A. This is not clear but the Ontario Ministry of Natural Resources recommended this instead of lethal sampling of fish.

C. Community studies are harder to design and execute than studies on single sentinel species.

Q. Can't we do an IBI (index of biotic integrity)? This is simple and fast to do.

Q. Do we want to examine the presence and use of habitat by fish or an IBI or what?

A. We are just asking for alternatives, whatever the EWG decides those are, because the option is dropping the AFS and this is unacceptable.

C. We hardly know enough biology of individual sentinel species, let alone enough to make judgements on whole ecosystems through community surveys.
Q.If we can't catch enough of one sentinel species, how will we sample an entire community?
A. In many cases sufficient sentinels were caught but killing them, and bycatch, is the problem.
C. Remember that community assessments will require adding replicate areas.

4. THE USE OF BIVALVES AND CRUSTACEANS AS SENTINEL SPECIES FOR THE EEM ADULT FISH SURVEY

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It has become evident that finfish have not been as suitable a sentinel for the AFS of the Pulp and Paper Industry Environmental Effects Monitoring program as initially thought. Consequently, there has been renewed interest in selecting marine invertebrates for use as sentinel species. A recent literature review of invertebrates common to various substrate types throughout the Maritimes has led to the selection of two groups which offer the best selection of sentinel choices; bivalve molluscs and crustacean shellfish. The bivalve candidates for sentinel species include: the blue mussel, *Mytilus edulis*, the soft shell clam, *Mya arenaria*, and the American oyster, *Crassostrea virginica* and the crustacean shellfish species include: the rock crab, *Cancer irroratus*, the Jonah crab, *C. borealis* and the green crab *Carcinus maenas*. Although either group of invertebrates offer choices for candidate sentinel species; the bivalves are considered a better group from which to select a candidate sentinel species. This is due to a considerable number of advantages offered by their ecology, biology and how well each species satisfies the criteria presently used in the selection of a sentinel species by the AFS Technical Guidance Document. Specifically, all the bivalve species mentioned above meet a substantial number of the criteria which include, substrate exposure, food preference, food chain involvement, abundance, longevity, fecundity growth rate and age to maturation among others. In addition, these bivalve species are common throughout the Maritimes (except for the American oyster which does not occur naturally in Newfoundland) a factor which offers some continuity to the establishment of a general protocol which may be applied across the Maritimes as opposed to specific provinces or regions within provinces.

What to measure as well as an approach strategy to be followed are not trivial problems that must be addressed once a sentinel species has been selected. A tiered or hierarchical approach is preferred. Crucial to the strategy is the determination of gross changes that are statistically real. The study can then be further expanded to address the cause of the gross changes and how they are manifested within the organism. Relatively simple analytical procedures can be used to quantify gross biological changes such as shell growth and tissue growth. More complex procedures such as, measures of contaminant levels in tissues, histological examination of tissues and biochemical enzyme analysis could follow. However a detailed blueprint or strategic plan to follow has yet to be addressed. It is highly unlikely that one approach will surface as a common plan to EEM programs. It is more likely that each mill will develop their own strategy based upon a cost/benefit approach tailored to the guidelines and/or recommendations provided by federal agencies.

Questions, Answers and Comments
Q. Aren't there problems with multiple spawnings and measuring gonads etc. in bivalves?
A. Yes, you must know the biology of the species and population. Therefore preliminary work and multiple field trips will be required.
Q. How many trips do you think are necessary?
A. This would have to be decided with the client and experts.
Q. How ready is this for Cycle 2? Is it ready for monitoring now or does it need further research?

A. This will be an item for discussion a little later.

C. Global "mussel watch" has gathered lots of data on mussels which may be useful.

5. REFINED CAGED BIVALVE METHODOLOGIES AS PART OF EEM: CHARACTERIZING CHEMICAL EXPOSURE AND BIOLOGICAL EFFECTS

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Caged bivalve monitoring has demonstrated the ability to be a potentially powerful tool in the Environmental Effects Monitoring (EEM) program for the pulp and paper mills in Canada by employing simple, yet refined methodologies. A significant number of refinements to the caged bivalve methodology have been made in the last decade that have dramatically increased the utility for characterizing chemical exposure and associated biological effects in effluent monitoring. The ability to reduce the uncertainty associated with traditional methods in performing assessments of ecological risk through a routine biomonitoring program make caged bivalves a potentially powerful monitoring tool. Moreover, during the development of those refinements, standard, well-known, and well-established endpoints (e.g., growth and bioaccumulation) have been used by many different investigators, including those in Canada and the U.S. The net result has been that the approaches have been very cost effective.

Working Hypotheses

Three working hypotheses were developed as a way to critically evaluate whether caged bivalves might represent a reasonable alternative to the adult fish survey approach, particularly in areas whether the failure to collect sufficient fish has been most pronounced. The hypotheses are: (1) Tissue chemistry characterizes exposure better than water or sediment chemistry; (2) Tissue chemistry can be used to help predict potential environmental effects; and (3) Bioaccumulation and growth in bivalves can be used to help predict potential effects on fish. Emphasis will be placed on evaluating these three hypotheses.

Characterizing Exposure and Effects

Using caged bivalves in monitoring programs is particularly effective since the approach combines the experimental control associated with laboratory bioassays with the environmental realism of traditional field monitoring. This facilitates reducing the uncertainty commonly associated with traditional approaches. Caging bivalves along suspected chemical gradients allows the integration of exposure and effects over space and time. This is particularly important since characterizing exposure and effects has been problematic in many adult fish surveys. The ability to control the supply of test organisms in areas of concern and make synoptic measurements of well-established endpoints like growth (that have been related to population effects) make this method particularly attractive as an alternative or perhaps even a supplement to the adult fish surveys in EEM. The exposure-dose-response triad that has been developed emphasizes the importance of tissue chemistry in establishing links between the environment and the organism as well as between results from traditional laboratory tests and field monitoring .

Bivalve Sensitivity

Bivalves are particularly effective at quantifying exposure because of their ability to concentrate and integrate chemicals in their tissues. These estimates of biologically available chemicals are not possible even with thousands of water samples. There is a perception among many scientists that bivalves are not very sensitive to chemicals because they survive exposure to high concentrations of chemicals and accumulate high concentrations of those chemicals in their tissues. Recent studies however, have shown that bivalves are as sensitive or more sensitive to a variety of chemicals (including those in pulp and paper mill effluents) than standard test organisms such as daphnia, fathead minnows, midges, and amphipods in both freshwater and marine environments (Salazar and Salazar, 1997a,b). Growth is one of the more commonly measured endpoints and reduced growth rates have been related to adverse effects on bivalve populations.

Mill Monitoring History

Many caged bivalve studies have been conducted in Canada and other countries for monitoring mill effluents. In Finland, scientists have been using this approach for mill monitoring since 1984. Almost all previous studies were conducted in freshwater environments and only used the bioaccumulation endpoint. At a mill in Alaska where the U.S. EPA required caged bivalve monitoring, the same methodology was used as in the TBT studies (i.e., minimum size range about 5 mm, compartmentalized cages for individual measurements, large replication with 100 animals/replicate). These results show that PCDD/PCDF were significantly accumulated since both beginning-of-test and reference concentrations were significantly lower. Changes in whole-animal wet weight and end-of-test tissue weights proved to be the most discriminating growth endpoints in these studies and a statistically significant relationship was found between tissue concentrations of PCDD/PCDF and mussel growth.

Linking Mussel and Fish Dose-Response

McCarty (1987) recalculated a series of QSAR regressions and combined them with other bioconcentration relationships to predict water concentrations for acute and chronic effects of organic chemicals on fish (Figure 5.1). Although there were seven orders of magnitude difference in the acute and chronic QSARs, there was only one order of magnitude difference between tissue concentrations predicted for acute and chronic effects. Donkin et al. (1989) measured water and tissue concentrations and mussel feeding rates to make similar predictions (Figure 5.2). These results suggested that tissue burdens would be a better predictor of effects than water concentrations. McCarty and Mackay (1993) have also suggested that using body residues as a reference framework could enhance ecological risk assessments. Collectively, these and other studies demonstrate the following: (1) The ability to predict tissue concentrations associated with effects in mussels, fish, and other species (existing data for TBT also support this conclusion for a variety of species). (2) The ability to predict dose-response relationships in fish from dose-response relationships in mussels. (3) Tissue burdens may be a better predictor of effects of hydrophobic organic chemicals than concentrations measured in either water or sediment. Another link between mussels and effects on fish could be established by using the P450 RGS (reporter gene system) to simulate effects in fish. Methods have been developed using a human liver cancer cell line engineered to produce luciferase instead of P450 (Anderson et al., 1995). If chemicals associated with mussel tissues are exposed to this test system, they can be used as a surrogate for the MFO induction that has been used in other adult fish surveys as an indicator of exposure and the potential for adverse effects. Most of the exposure and effects endpoints that have been measured in fish can be measured in bivalves.

Prospectus

Having demonstrated the utility of various relationships between exposure and effects in previous mill monitoring (Salazar & Salazar, 1997a,b) it appears as though caged mussels could be used as an alternative to adult fish in Cycle II in EEM. Beyond that, the use of caged bivalves in concert with fish exposures could be used in a preponderance-of-evidence approach to demonstrate exposure to pulp mill effluents and associated effects. Caged bivalves could be used for obtaining other useful information as well. (1) Caged bivalves could be used to map and monitor the exposure plume more efficiently than traditional approaches because of their ability to quantify bioavailable chemicals. (2) Caged bivalves could be used to validate laboratory bioassay studies and confirm that similar exposures and effects have been demonstrated in the lab and the field. (3) In addition to evaluating the results of the adult fish survey, caged bivalves could be used to evaluate the relative effectiveness of both laboratory bioassays and benthic community assemblages in monitoring mill effluents. It is important to note that in the Alaska pulp mill study, significant accumulation was found in caged bivalves while laboratory exposures with contaminated sediment did not show significant accumulation in clams. Surprisingly, the relationship between tissue burdens in mussels and sediment used in the laboratory bioassays with clams was significantly better than the relationship between clams and the sediment to which they were exposed. These results suggest that field bioassays with caged bivalves in the water column are a better predictor of exposure to mill-associated chemicals than laboratory sediment bioassays with infaunal bivalves.

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Questions, Answers and Comments

Q. Are effects on reproduction easy to measure? How?

A. Someone else did this but it's hard to interpret. Others who are more proficient do it better. It is usually done by measuring the weight of the gonad.

Q. What would be the cost for a pulp mill study without the bioaccumulation study?

A. $8-10K US for the initial setup, i.e., consultants to come, teach, and run the experiment. Then, $15-20 K if two people are needed, $40K if a whole team of consultants do the whole thing.

Q. How do you deal with confounding point sources?

A. Spatial resolution is fine with this technique by gradient studies away from each source. However, if two different effluents are exactly coincident, then no technique can resolve their separate effects. This technique is ready for Cycle 2 and its your best bet.

Q. How euryhaline are these bivalves, will this work in the estuarine environment?

A. Different species will be required for different environments and there are data available to help in making selections. There is a mountain of data supporting use of tissue residues; different species are in some cases comparable in this aspect.

Q. What chemicals should we measure in bivalves exposed to pulp mill effluents?

A. I don't know but whatever you choose you'll find it concentrated by mussels. Perhaps Kelly Munkittrick could offer advice.

C. There are 5 or 6 compounds in pulp mill effluent which have been associated with biological responses but there are probably dozens or even hundreds of other chemicals that have not yet been identified. In any case though, bioaccumulation is not the point of the fish survey aspect of EEM, we really only need look at biological effects.

C. True, but even without knowing which chemicals are responsible for whatever biological effects are observed, we could measure a series of chemicals with different bioaccumulation characteristics or KOWs so we at least know the range.

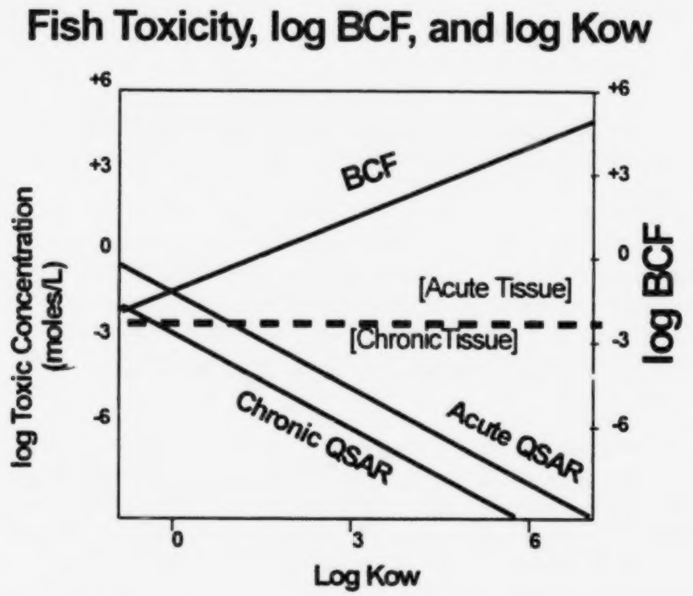

Figure 5.1. The relationship between fish toxicity, log BCF and log Kow. Note how although acute and chronic QSARs span seven orders of magnitude along with BCF, tissue burdens associated with acute and chronic toxicity are constant and separated by about one order or magnitude. (Redrawn from McCarty, 1987)

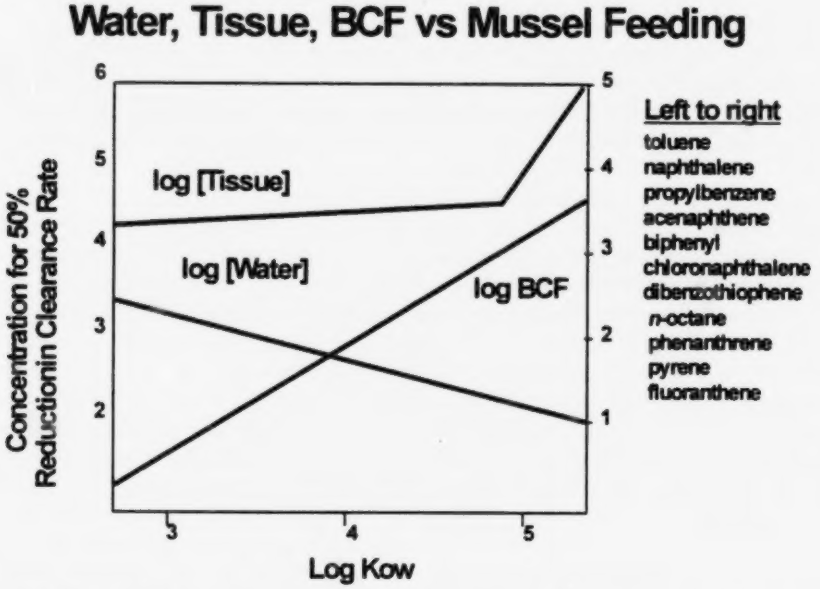

Figure 5.2. The relationship between mussel feeding, log BCF and log Kow. Note how although the water concentration and BCF associated with reduced feeding spans several orders of magnitude, tissue burdens associated with reduced feeding are nearly constant over a wide range of log Kow. These relationships are very similar to those shown for fish in Figure 1. (Redrawn from Donkin et al., 1989.)

6. HABITRAP: A BIOMONITORING SYSTEM USING BIVALVES FOR ASSESSING ENVIRONMENTAL IMPACTS FROM CHRONIC POLLUTION.

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A Biological Monitoring System, called HABITRAP, was previously developed in response to a need for more practical, sensitive and easily interpreted biological effects indices that can be used in offshore oil and gas Environment Effects Monitoring (EEM) programs. A similar need exists for alternative approaches to pulp and paper mill EEM. The system uses bivalve filter feeders as sentinal organisms for environmental impacts as they are: abundant and widely distributed; commercially and ecologically important; unable to escape pollution; able to process large volumes of water; and can be used to indicate both the biological availability and consequences of a wide range of pollutants.

The most important biological responses of organisms to pollution stress are those that affect their growth, reproductive potential and survival. This is particularly true for a resource species. Tissue growth is clearly related to population effects as it determines the organisms ability to contribute to the gene pool. Pollution stress does not act directly on growth, but on the biochemical and physiological processes controlling growth. Numerous studies have shown that bivalve growth responses to a wide variety of contaminants depend primarily on changes in processes controlling energy acquisition rather than energy utilization. Bivalve feeding behaviour is therefore a sensitive index of the organismal and population effects of contaminants. The HABITRAP Biological Monitoring System is based on the measurement of bivalve feeding responses to ambient changes in environmental and contaminant conditions.

The HABITRAP provides continuous measurements of the clearance, ingestion, absorption and egestion rates of a population of bivalves held *in situ*. The method has been extensively tested, and the results are comparable to those obtained using traditional laboratory methods. However, as the measurements are obtained under conditions of natural food supplies, flow conditions and contaminant levels, the *in situ* responses of bivalves are more reliable than laboratory measurements. The HABITRAP method has the additional advantages of being largely automated and relatively inexpensive.

Bivalve growth rate is a scientifically defensible and useful index of water quality. However, there are two major advantages to measuring bivalve feeding behaviour in EEM programs as a combined index of growth and environmental stress. First, continuous monitoring of feeding rate permits an assessment of the relative impact of short- and long-term environmental changes. Short-term events of importance may include large changes in effluent discharge composition and rate or the resuspension of previously deposited contaminants during storms. Direct growth measurements can only indicate the cumulative stress response over a period of several weeks to months. The ability of the HABITRAP to identify the relative impact of different industry activities would provide regulatory agencies with information on the best practices for the pulp and paper industry to follow for minimizing impacts. Second, feeding behaviour measurements provide a physiological explanation of the mechanism of population impacts and, if used under well defined conditions, permit a diagnosis of the relative impact of natural and anthropogenic stressors. An increased knowledge of cause-effect relationships would enhance our capacity to predict the biological effects of known contaminants.

Questions, Answers and Comments
Q. How do you get ingestion rate from egestion rate?
A. Animals don't absorb any ash so egestion of ash = ingestion rate of ash.
Q. How do you see applying this to Cycle 2?
A. I prefer a gradient approach to an impact vs reference site approach.
Q. Is this ready for Cycle 2?

A. No. The HABITRAP is already developed and proven for offshore oil development monitoring but you would need a pilot project to adapt this for monitoring pulp and paper mill effluents. Therefore, this development should be done during Cycle 2 to provide the tool for standard use in Cycle 3.
Q. What would the cost be?
A. Approximately $2K per automated HABITRAP.
Q. What number of animals do you use per collector?
A. Ten scallops or 25 mussels.

7. DEVELOPMENT AND TESTING OF A MESOCOSM TECHNOLOGY FOR ENVIRONMENTAL EFFECTS MONITORING (EEM) METHODS APPLICATION

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Mesocosms refer to field-based artificial stream systems which can be used in aquatic Environmental Effects Monitoring (EEM) programs as a tool to assess the effects of industrial and municipal effluents on aquatic biota. Typically, the effects of effluents on aquatic receiving environments are assessed through field monitoring programs or effluent sublethal toxicity tests. In field monitoring programs, water chemistry, benthic invertebrates and/or fish populations are sampled from sites exposed to effluents and these results are compared to samples collected from reference sites. In some cases results from field monitoring programs are inconclusive due to the high natural variability of sampled populations, inadequate reference sites for comparison to exposed sites, lack of understanding of the level of exposure, the presence of confounding effluent discharges, and/or inadequate statistical replication (pseudo replication). Sublethal toxicity tests are laboratory testing procedures where the effects of an effluent are assessed on single species under controlled laboratory conditions. The significance of sublethal toxicity test results to the natural environment can be difficult to determine as these tests lack realism, and in many cases use test species which are not endemic to the area being studied. Artificial streams can provide an integral link between field monitoring programs and sublethal toxicity testing by simulating the natural environment while allowing for variable control, experimental manipulation, statistical replication, and most importantly, providing cause-and-effect results to determine the effects of specific effluents on aquatic biota. Artificial streams are not intended to replace field monitoring programs or sublethal toxicity tests but are intended to be used as an assessment tool in conjunction with these other EEM methods to strengthen the testability of hypotheses and allow for site-specificity in EEM program design.

An artificial stream system was developed by the National Hydrology Research Institute (NHRI) of Environment Canada in Saskatoon, Saskatchewan to determine the effects of pulp mill effluent on algal and benthic invertebrate communities in freshwater rivers in Prince George, British Columbia and Hinton, Alberta (Culp et al. 1996, Culp and Podemski 1995). This system consisted of a total of 16 circular tanks (diameter 1 m) on two portable trailers. These trailers were set up beside a reference site and ambient water was pumped into a head tank, through a distribution manifold, and then delivered to each stream at a controlled rate (2 L/min). Water depth within each stream was controlled at 25 cm by a central overflow standpipe. This depth and flow rate resulted in a hydraulic residence time of 2 hours in each stream. Effluent was delivered independently and continuously to individual streams using peristaltic pumps. Current velocity within each stream was controlled at 20 cm/s using a belt-driven propeller system. Each stream was then "seeded" with natural substrates, algae and benthic invertebrates endemic to that river being studied. Experiments were conducted for approximately 4 to 6 weeks and biological end-points measured to determine the effects of the effluent on algal growth, composition, and benthic invertebrate biomass, density, and community structure. In the Prince George, Fraser River studies three treatments were created; control (no effluent), 1% effluent ("zone-of-influence"), and 3% effluent. Results showed that, at these effluent concentrations, treated bleached kraft pulp mill effluent increased algal biomass (enrichment effect) but did not affect algal community composition (Culp et al. 1996). In the Hinton, Athabasca River studies three treatments were created; control, 1% effluent, and 1% nitrogen + phosphorus (at levels found within 1% effluent) (Culp et al. 1996). The objective of this latter experiment was to decouple the effects of nutrients and contaminants within treated bleached kraft pulp mill effluent. Results showed that the effects of the effluent were restricted to nutrient enrichment and caused an increase in algal biomass, benthic invertebrate growth and total benthic invertebrate abundance relative to the control streams.

Mesocosm systems are a suitable monitoring alternative which should be considered for future cycles of the pulp and paper EEM program. The results of the first cycle of the EEM program were submitted by pulp and/or paper mills on April 1, 1996. A review of the first cycle results by Environment Canada and the Department of Fisheries and Oceans, revealed that several program inadequacies existed particularly regarding site specific flexibility in program design, control over environmental variables being monitored, the adult fish survey (AFS), sublethal toxicity testing and EEM programs conducted in marine (estuarine) receiving environments. The primary problems with the AFS were related to the migratory habits of the selected sentinel species, the complexity of effluent plume dispersion and lack of suitable tracers to confirm exposure. Problems identified with the sublethal toxicity testing component of the EEM program included insensitivity or over-sensitivity of test organisms, use of organisms not indigenous to receiving waters, and use of water not similar to local receiving water.

The artificial stream system developed by the NHRI has been effective in assessing the effects of effluents on algal and benthic invertebrate communities in freshwater receiving environments. This system has the potential to assess the effects of pulp mill effluents on fish, particularly forage species. This system could also be re-designed for use in marine (estuarine) environments. To explore these applications further, Washburn & Gillis Associates Ltd. and the NHRI have entered into a collaborative research and development project to re-design the existing artificial stream system and to test and evaluate its effectiveness (technical and cost) as a tool for subsequent cycles of the EEM program.

A phased approach has been designed to meet the project objective. This approach will consist of four phases: 1) stream system design and client requirement assessment, 2) stream system construction, 3) system testing (physical and field), and 4) system evaluation and potential implementation. Results from the first phase will form the basis to plan subsequent phases. Proposals for funding will also follow this phased approach. Funding to complete Phase 1 of the project has been obtained by Washburn & Gillis Associates Ltd. and the National Research Council's Industry Research Assistance Program (IRAP). Phase 1 will be completed by the end of February. Proposals are currently being prepared to obtain funding for subsequent phases of the project. It is anticipated that Phase 2 (construction) will commence in March or early April, Phase 3 in June or July, and Phase 4 in September. Full evaluation of the effectiveness of this artificial stream technology as a tool for EEM programs should be completed by December 1997. A successful evaluation of this mesocosm technology requires the cooperation and participation of industry, Environment Canada and the Department of Fisheries and Oceans to ensure their respective concerns and questions are addressed in this research and development project.

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Questions, Answers and Comments

Q. You need input from BC mills for a west coast perspective. Have you looked into this?

A. Not yet.

Q. There may be problems with suspended particulates. Are your flows high enough to handle levels seen in nature?

A. In Fraser and Athabasca River tests, yes. We got some settling out of sediments in pipes but turbidity etc. in our tanks was still comparable to what was seen in the river. This may depend on particle size with large particles, like sand, being a problem. This will need to be looked at, especially given the importance of particulates in pulp mill effluents.

C. Levels as high as 3000 mg/l suspended material have been recorded in BC rivers so this certainly is an issue.

Q. What test duration will you use and what endpoints will you look at in your 1997 pilot study?

A. Four to 6 weeks duration. Endpoints will include MFO, tissue chemistry, reproductive hormones, liver and gonad weight and growth.

Q. What would be the cost of this approach?

A. The initial cost of the setup would be approximately $80K. Thereafter, it should be approximately $20K to run an experiment, depending on which endpoints are measured, length of exposure etc. You don't necessarily have to monitor every day.

Q. Is this ready for Cycle 2?

A. This approach has been used for bacteria, invertebrates and algae but not fish and not in the estuarine and marine environments yet. The technique is ready for a pilot project in the summer of 1997 and then application in Cycle 2.

Q. So the mesocosm approach follows field surveys, asking more refined questions, rather than replaces the field survey?

A. No one is suggesting that mesocosms replace field or lab studies, they complement them. Where field studies cannot be carried out, mesocosms provide an alternative. Where field studies can be carried out, you might look at a tiered testing approach with field monitoring and lab bioassays first and mesocosm work afterwards to nail down effects of effluent.

8. THE APPLICATION OF THE SCOPE FOR GROWTH APPROACH TO ASSESS ENVIRONMENTAL IMPACTS FROM PULP MILL EFFLUENTS AND OTHER POINT SOURCES

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"Scope for growth" (SFG) describes the "excess" energy accumulated by an organism after its current energy demands are met, and which is usually directed to somatic growth or reproduction. The rationale for using SFG to monitor environmental quality is that an organism under stress will use energy differently from one not stressed; this difference in energy partitioning is reflected in SFG. Two points about SFG should be obvious: first, stress does not necessarily mean "pollution". In fact, SFG could reflect any change to ambient conditions which results in the organism's processing energy differently; this could include physical disturbances, temperature changes, or changes in food supply, etc. Second, SFG can, in principle, be measured in any organism, though in practice it has been used most in sessile bivalves, where energy balance is not distorted by variations in energy expenditure during movement.

One might ask why growth itself, rather than SFG, is not measured. The answer is that SFG can be measured over a period of a few hours and under controlled conditions, whereas measurements of growth in organisms such as molluscs require at least a few days, or, more usually, weeks; during this interval, sources of stress can change. However, examples exist of changes in molluscan growth being ascribed to a single pollution source (e.g., Black, 1973).

The simplest approach to measuring SFG is to take organisms from their natural habitat and measure feeding, respiration and excretion rates over a few hours under controlled conditions. SFG may then be calculated from the equation:

$$\text{SFG} = \text{Energy absorbed} - (\text{energy respired} + \text{energy excreted})$$

where energy absorbed is measured via filtration or clearance rates (for filter feeders), respiration rate is measured by oxygen consumption, and excretion is estimated from ammonia production (from molluscs) or other forms of nitrogen excretion.

Numerous field studies have illustrated the use of this approach to assessing the impact of pollution. Martin et al. (1984) found SFG in mussel *(Mytilus edulis)* collected from various points in San Francisco Bay to be negatively correlated with several metal and organochlorine concentrations in transplanted *M. californianus*, which was used as a "bioaccumulator" of contaminants. In a similar study, SFG in *M. edulis* from sites along a contamination gradient in an industrialised fjord in Norway declined gradually with increasing contamination by PAH, PCB and (less clearly) metals (Widdows and Johnson, 1988). In a pollution gradient in Hamilton Harbour (Bermuda) a local bivalve *Arca zebra* was transplanted from "clean" sites and caged for 11 - 12 d at suspect sites; this was long enough for contaminants to accumulate through feeding and for the physiological responses to develop. Declines in SFG at contaminated sites were well correlated with increasing tributyltin (TBT) concentrations in the water column and with tissue concentrations of PAH and Pb (Widdows et al., 1990). Sewage discharges may also reduce SFG: at a sewage sludge dump site off Plymouth (UK) transplanted *M. edulis* had lower SFG than animals caged inshore (Lack and Johnson, 1985). The bivalves *M. edulis aoteanus* and *Perna canaliculis* sampled from close to a sewage outfall at Wellington, NZ, had lower SFG, and higher metal concentrations, than those from further away from the source of pollution (Anderlini, 1992). In cockles (*Cerastoderma edule*) from various sites in Southampton Water (UK) SFG (and other indices of health) were generally related to the level of contamination of the site with metals and hydrocarbons (Savari et al., 1991). *M. edulis* caged in the expected pollutant "plume" from an industrial discharge area on the west coast of Sweden had lower SFG than animals caged at a "reference" site (Magnusson et al., 1988). Changes in SFG also explained variations in growth rate of mussels *(M. galloprovincialis)* reared at different sites in an estuary used for aquaculture, though in this case the SFG changes reflected quality and quantity of food, rather than contamination (Navarro et al., 1991). Measurements of SFG are not restricted to bivalves: the invertebrate *G. pulex* showed reduced SFG (resulting from reduced energy assimilation) at a polluted site as compared to a reference site (Maltby et al., 1990). Often, the most obvious impact of stress --- whether from pollution or other causes --- is on the clearance or filtration rate component of the SFG equation (Maltby et al., 1990; Anderlini, 1992; Okomus and Stirling, 1994). Table 1 summarises some of the impacts of pollution on SFG in bivalve molluscs.

The measurement of SFG over the short term can easily be related to longer-term changes in growth rate or condition (Savari et al., 1991; Magnusson et al., 1988; Navarro et al., 1991). SFG is therefore attractive as an early warning of potential long term effects.

No "field" studies appear to have been carried out on which the response of SFG to pulp mill effluents. In one lab. study (Sunoko, 1993) *Mytilus edulis* were exposed during 7 day static bioassays to effluent from a pulp mill discharging into Boat Harbour, NS. SFG was reduced significantly in *M. edulis* exposed to 40% v/v effluent in sea-water; the reduction was due to reduced filtration rate by the test animals. Concentrations of effluent greater than 50% were lethal to mussels so could not be tested; 20% effluent had no significant effect on SFG.

There seems to be no reason why SFG should not be used as a field measure of the impact of pulp mill effluents on appropriate "indicator" sessile bivalves.

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Table 8.1. Some examples of changes in Scope for Growth (SFG) in bivalves in response to pollution stress

Site, Species	Reference	Response
San Francisco Bay, CA (sewage influence) *Mytilus edulis* (resident)	Martin et al. 1984	Decline in SFG with increasing chlordane, dieldrin, Cr, Cu, Hg, Zn, Ag, Al concs.
Industrialised fjord, Norway *Mytilus edulis* (resident)	Widdows and Johnson 1988	Decline in SFG with increasing PAH and PCB gradient
Southampton Water, UK (mixed contaminants); *Cerastoderma edule* (resident)	Savari et al. 1991	Decline in SFG correlated with increasing Cu in sediments
Hamilton Hbr. Bermuda *Arca zebra* (transplanted 11-12d)	Widdows et al. 1990	Decline in SFG with increasing PAH and TBT gradient
Wellington Harbour, New Zealand (sewage impact) *Mytilus edulis aoteanus* *Perna canaliculus*	Anderlini 1992	Decline in SFG with increasing tissue Ni conc.

Questions, Answers and Comments

Q. At what point is it more sensible to measure actual growth than SFG?

A. It's a question of rapidity; SFG is much quicker - virtually instantaneous - to measure. It depends on what question you're asking.

C. Sure, SFG is faster than looking at growth in caged bivalves but it gives poorer information.

C. SFG is easy to measure but hard to interpret. Bivalves have a great ability to acclimate to conditions so you must know your biology. SFG might be a better replacement for the benthic invertebrate survey than for the fish survey.

Q. How many animals are tested in a SFG study?

A. Widdows generally used 8-10 animals per treatment.

Q. Will this be too expensive? Who will do the work?

A. For a pilot project, a graduate student. One idea is to request 40-50K to get a graduate student to look at SFG as an indicator of general health of 6 coastal sites in BC.

C. If SFG is appropriate for graduate theses, then its a research project and not appropriate for EEM. If it's not research, then a graduate student shouldn't get a degree for doing it. Therefore graduate students are not an answer to cost effectiveness.

C. SFG is a general response to environmental conditions, not specific to pulp mill effluent. Do we want this for EEM or specific responses?

C. Shouldn't we be putting together a suite of endpoints rather than discussing which is best?

9. STATUS REPORT FROM THE FISH SURVEY EXPERT WORKING GROUP

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The amendments to the *Fisheries Act* passed in May, 1992, included a provision for Environmental Effects Monitoring at pulp and paper mills in Canada. The first cycle reports were due on April 1, 1996. An Expert Working Group (EWG) was formed to review the results of the fish survey component, with representatives from government, the pulp and paper industry and consultants with specialized expertise. The main purpose of the EWG was to review the first cycle objectives and to make recommendations for the second cycle.

The EWG reviewed 112 studies submitted, only 7% of the studies were determined to have been totally successful, and 53% were unsuccessful. The remainder were partially or marginally successful based on numbers of fish caught. The EWG documented generic problems faced during the completion of the Cycle One studies. In marine and estuarine systems, the dominant problems were related to migratory fish and an inability to document exposure. No marine and estuarine surveys were totally successful. In freshwater, the dominant problems were related to an inability to catch fish, uncertain exposure, problems with reference sites, the presence of confounding factors and incomplete or poor reporting of data. The data were reviewed as they applied to the secondary objectives of the first cycle: evaluating species suitability, collection methods and reference sites. A review of variability showed large differences between studies, even those at closely related sites.

It was determined that the generic survey required in the first cycle was not appropriate for all receiving waters, and a decision tree was developed to assist in determining when an alternative approach was warranted. It is felt that the decision tree will be useful for industry, their consultants and the local authorities in defining a suitable study design for cycle two on a site-specific basis. Alternative approaches are required in situations where fish were not present, not exposed or the data collected could not be interpreted. The present workshop is the EWG's opportunity to review alternative approaches with regard to their readiness for inclusion in Cycle 2. Approaches deemed promising but not yet proven might be explored on a pilot scale where acceptable to both the mill involved and the regional technical advisory panel.

The EWG will submit a draft report to the National Technical Management Committee (TMC) at the end of April 1997. Following this, it is expected that the EWG will continue work on sections not completed, pending the comments and advice of the TMC. Included in the final report will be a series of recommendations for research and development needs for the fish survey component of EEM.

Questions, Answers and Comments

Q. Has the EWG determined what effect sizes should be targeted?

A. The EWG has discussed gonad sizes. In our previous research we have found 25% changes in fish exposed to pulp milleffluent. The EWG is presently looking at variances seen in reference sites.

Q. With regard to alternative approaches, what criteria will be used to determine which alternatives are suitable?

A. The EWG will be discussing criteria immediately following this workshop. From that we also expect to generate a list of research needs.

C. We should include all potential approaches at this stage.

C. We should keep in mind Richard Addison's point that we should be looking at commercially important species.

C. Perhaps, but we don't care only about commercially important species, we care about the effects of pulp mill effluent on the entire biological integrity of the receiving environment.

C. This brings us back to the definition of exactly what question the fish survey is supposed to address which has never been clear.

10. HEALTH ASSESSMENT OF FINFISH COLLECTED FROM ESTUARIES RECEIVING PULP MILL EFFLUENT

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Finfish collected from estuaries in the southern Gulf of St. Lawrence which receive pulp mill effluent were examined to detect fish health bio-markers that could be used to create an index for evaluating the impact of pulp and paper mill effluents on finfish populations. Sampling was conducted in five estuaries located in Nova Scotia or New Brunswick, three of which receive pulp mill effluent (Restigouche River, N.B., Miramichi River, N.B. and Pictou Harbour, N.S.) and the remaining two sites were designated as reference sites (Margaree River N.S. and Kouchibouguac River, N.S). Fish were collected for examination in both the spring and fall of 1996 and consisted of Atlantic tomcod (*Microgadus tomcod*) and flounder (*Pleuronectes americanus* and *P. putnami*), predominately.

All fish were examined for gross abnormalities. A wide variety of media (tryptone soya agar (TSA), blood agar (BA), marine agar, cytophaga agar (CA), Shieh's agar (SH) and *Flexibacter marinus* media, as well as TSA, BA , CA and SH all supplemented to 2% salt content) were used to detect the presence of bacterial agents in major organs and skin lesions. Tissue samples (kidney, spleen, gill and pyloric caeca) from all fish were assayed to detect viral agents using the Canadian Fish Health Protection Regulations (FHPR) virus methodology and a variety of cell lines (chinook salmon embryo, grunt fish and epitheloma papulosum cyprini). All abnormalities detected were forwarded to the Atlantic Veterinary College (AVC) in P.E.I. for a histological examination. During the fall sampling only, liver and gill samples collected from 25 Atlantic tomcod from each site were examined for any abnormal pathology and the presence (i.e., intensity and prevalence) of parasites by AVC.

Gross deformities and other abnormalities were detected in low frequency from both the estuaries receiving pulp mill effluent and the references sites. There were no viruses identified using culture techniques. Although a variety of bacterial species were isolated, with the exception of the pathogen atypical *Aeromonas salmonicida*, isolated from Atlantic tomcod from Miramichi River (spring sampling) and Pictou Harbour (fall sampling), all isolates were the typical, ubiquitous bacteria expected from estuarine/brackish water environments. A variety of parasites were identified either as encysted intermediate life stages or as adults (final hosts) and none were considered as abnormal for the fish species examined.

Unfortunately there were no specific bio-markers of exposure to pulp mill effluent identified with which to formulate a Fish Health Assessment Index. Potential disease agents were identified in both reference and pulp mill sites. Statistical analysis is presently underway to determine whether the incidence of parasites and other pathological conditions differs between estuaries with pulp mills and without. If differences in the expression of disease are found between estuaries, a potentially useful direction for future research will be an examination of the role of immunosuppression in finfish populations exposed to pulp mill effluent.

Questions, Answers and Comments

Q. Did you always get your sample size of 60 fish per site?

A. No, but we did for tomcod.
Q. Did you age fish or a representative sample?
A. Yes for tomcod, which were all 2-4 years old, but other species were not aged.
Q. Are there any data in the literature relating effects that you found to pulp mill effluent?
A. Yes, but most studies were laboratory exposures, not field studies like ours.
Q. Were the fish you sampled actually exposed to pulp mill effluent plumes?
A. Tomcod and winter flounder move around estuaries and the coastal environment a lot and present an integrated signal of contamination in that scale of area. Mummichogs, for example, are less mobile and may better reflect the effects of one particular point source.
Q. So you don't have any direct exposure data at all for your fish?
A. No. But we know from others'work that with a high enough dose of pulp mill effluent you can get some of these effects.
C. You can get such effects with a high enough dose of anything. You would need concurrent lab and field studies to tie health effects of pulp mill effluent.
Q. There is extra stress on fish during spawning which may result in a greater expression of disease, e.g., frequency of lumps and bumps. Did you sample during spawning?
A. No.
Q. With the data you collected, can you do a Health Index on the fish?
A. Yes, and we will be doing this on samples of adequate size.

11. USE OF A FISH HEALTH ASSESSMENT INDEX APPROACH FOR EVALUATING IMPACTS OF MULTIPLE STRESSORS - A WEST COAST PERSPECTIVE.

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A number of fish health and chemical contamination problems have been documented in mountain whitefish, *Prosopium williamsoni*, caught in the Columbia River between the Hugh Keenleyside Dam and the International Border, in British Columbia. Abnormalities, such as external lesions, discoloration, and thin fish were first reported by field biologist working on the river in October 1990. A consumption advisory was also in effect for this species from June 1990 to March 1995 due to dioxin contamination. A study conducted by DFO in January 1991 (Boyle *et al.* 1992) suggested that the health of mountain whitefish may be impaired.

The three main stressors on this stretch of the Columbia River are: the bleached kraft pulp mill located near Castlegar, B.C.; the lead/zinc smelter and fertilizer plant at Trail, B.C.; and numerous dams and run-of-the-river reservoirs on the Columbia River system. The Department of Fisheries and Oceans (DFO) conducted a monitoring program in this area to document the recovery of the mountain whitefish following the expansion and upgrading of the pulp mill, and the ongoing process changes at the smelter and fertilizer complex. Pulp mill improvements included 100% chlorine dioxide substitution for chlorine in the bleaching process and an air-activated sludge secondary treatment system. The smelter implemented several projects, which significantly reduced the loading of mercury and other metals to the Columbia River. B.C. Hydro also made changes in relation to facility operation to reduce dissolved gas supersaturation in the Columbia River downstream of the Hugh Keenleyside Dam.

The study consisted of three sampling events, in July of 1992, 1994, and 1996. Adult mountain whitefish between 25 to 40 cm in length were collected from two sites on the Columbia River, at Genelle, approximately 10 km downstream of the pulp mill, and at Beaver Creek, about 12 km downstream of the smelter, and from a reference site on the Slocan River. The total number of fish analyzed for wet weight, fork length, age, and disease survey (i.e., gross external and internal examination, bacteriology, and histopathological examination of gill, liver, spleen, kidney and pyloric caeca/hindgut) was approximately 60 fish per site. A subset of fish at each site were analyzed for dibenzodioxins and dibenzofurans (PCDD/PCDF) and polychlorinated biphenyls (PCBs) in muscle tissue (n=10), and mixed function oxidase (MFO) activities (n=15). Another subset of fish was analyzed for metals in muscle tissue, and metallothionein (n=15). All fish analyzed for chemical contamination or biochemical indicators were also analyzed for stomach contents. A separate set of 10 fish from each sampling location were analysed for parasitology, age, and size.

A cumulative disease severity (CDS) coding system was developed as a method of interpreting the fish health significance of the disease survey results, and quantifying the cumulative effect of all abnormalities present in one fish. The coding system subjectively rates the various types of abnormalities as to their potential impact on fish health. An abnormality such as a parasite cyst was rated light (1), reflecting the impact it is likely to have on fish health. The impact of causative organisms such as myxobacteria was considered to be moderate (2), while the finding of mycobacteria is considered more damaging, and was rated as severe (3). The normal condition as well as any artifacts of sampling or slide preparation were given a rating of (0). The total numerical value of all abnormalities found in one fish at a given location was used to indicate the CDS or health status of that fish. This rating system is based on the quantitative autopsy-based system developed by Adams *et al.* (1993).

Results from the July 1992 sampling phase of this study are documented in Nener *et al.* 1995. In 1992 fish sampled below the pulp mill at the Genelle reach exhibited consistently higher frequencies of abnormalities and significantly greater disease severity than fish sampled from the Beaver Creek reach of the Columbia River, located downstream of the smelter, or the reference site on the Slocan River. Fish sampled from Genelle were also significantly older than fish from Beaver Creek or the Slocan River, and a significant relationship between age and CDS was found at both Genelle and Beaver Creek. The confounding effects of age made it difficult to determine whether the greater frequencies of abnormalities and disease severity in fish from Genelle was related to natural ageing or other environmental conditions. PCDD, PCDF and PCB concentrations were also higher at the two Columbia River sites than at the reference site in 1992. Disease incidence and severity could not be correlated with organic contaminant concentrations. Fish sampled below the pulp mill in 1992 had significantly higher muscle concentrations of mercury than fish from either Beaver Creek or the Slocan River, but all values were below the federal human health consumption guideline.

Results of the 1994 sampling program are documented in Antcliffe *et al.* 1997. In 1994 male and female mountain whitefish sampled from the Genelle and Beaver Creek reaches of the Columbia River had significantly higher condition factors, size-at-age, and gonadosomatic index (GSI) than the similarly-aged fish from the reference site. Liverosomatic index (LSI) was higher in female fish sampled at Genelle, downstream of the pulp mill. The presence of loose eggs in the body cavity of 29% of fish from Beaver Creek and 7% of fish from Genelle may indicate nonspawning with resorption of mature oocytes, possibly in response to stress. PCDDs/PCDFs in muscle tissue declined substantially since the 1992 study, and the consumption advisory for this species was lifted based on these data.

In 1994 mountain whitefish sampled from the Genelle and Beaver Creek reaches of the Columbia River had consistently higher prevalence and severity of abnormalities when compared to the reference site on the Slocan River. Mean CDS was also significantly higher at Genelle than at Beaver Creek or the Slocan River reference site. However, abnormalities commonly associated with exposure to pulp mill effluent or metal contamination were observed at low levels, and several abnormalities appeared to be stress-related. CDS was also not correlated with organic contaminants or MFO activity. Results suggest that the CDS rating likely reflects the cumulative effect of all stressors on the system, including effluent discharge from the pulp mill and smelter, and flow regulation.

Results from the final 1996 study are preliminary, however, they appear to be consistent with those found in 1992 and 1994. Recommendations for further study include assessment of effects of various stressors (e.g., pulp mill effluent) on immunosuppression.

The fish health assessment approach used in this study provided a useful screening tool for evaluating the cumulative effect of all stressors on the health of Columbia River mountain whitefish. In general, application of fish health assessment should be based on site-specific needs. Recommendations for conducting fish health assessment include:

- Evaluation of approaches to determine which health-related variables are worth examining;
- Development of a protocol for field methods, including consistent terminology and practitioner training;
- Examination of summary indices and key abnormalities, as single number indices can mask spatial and temporal changes in individual tissues or organs;
- Tiered assessments, starting with gross external and internal examination followed by more detailed histology, bacteriology, and parasitology, if required;
- Similar age distributions among sampling locations to minimize confounding effects of age (older fish tend to have a higher natural incidence of abnormalities and disease);
- Assessment of natural background rates of disease and abnormalities, including natural variability, in order to interpret fish health assessment results;
- Definition of biologically important effects sizes for health-related variables.

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Questions, Answers and Comments

C. This study is a good example of a basin approach.

C. Although you can make an summary Index from these data, the real value is in comparing individual effects across sites.

C. We need to standardize our field crews, more training is needed to cut down on subjectivity.

Q. Were sites compared statistically?

A. Yes, means and standard deviations were calculated for each site. If confounding factors were significant we factored them into our model.

Q. Did you do an AFS-type survey too with the life history variables you collected, and did you leave enough time to see changes? Older fish may have tumours from long ago and improved effluent would not have made them go away.

A. The life history data are there and we're looking at them. With regard to timing of sampling, some workers feel that we should resurvey every year but I think that's too frequent.

Q. Has anyone looked at health indices in crabs?

A. Not to my knowledge.

C. In fact a health index has been worked out for blue crabs.

12. INVERTEBRATE PATHOLOGY ALTERNATIVES FOR MONITORING PAPER INDUSTRY EFFECTS ON ENVIRONMENTAL HEALTH

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Rationale for selecting invertebrate species to monitor environmental effects of pulp and paper mill ellfuent

Environmentally induced pathology in aquatic invertebrates is relatively undocumented (Cheng 1993) compared with that in aquatic vertebrates (Overstreet 1993; Harshbarger *et al.* 1993). Invertebrates lower in the food chain would be expected, however, to reflect to a greater or lesser degree the conditions of their environment. In addition, sedentary species would be expected to be especially sensitive to environmental changes which exceed those within the genetically-selected tolerance range of the organism. Species with shorter life-spans would also reflect relatively immediate changes in their microhabitats. Environmentally induced pathology can fall into several different categories, e.g., nutritional deficiencies (destruction of optimum or adequate food sources), genetically induced abnormalities (alteration of reproductive stability), environmental stresses (temperature, salinity, turbidity, oxygen deficiency, hydrogen sulphide excess, anthropogenic or biotic toxicity (e.g., pollutant chemicals or algal biotoxins), as well as others which currently defy identification (classic "unknown causes"). The well-known model of Snieszko (1973) can, therefore, be modified according to host susceptibility to environmental changes. Sedentary and short-lived, lower trophic level, species would show a greater overlap with the environment than longer lived, motile, species which can evade sub-optimal environmental influences. Environmentally influenced pathogenicity can thus be seen to have a greater impact on the former group of organisms. Consequently environmental pollutants have both direct and indirect effects on health. Differentiating between the two spheres of influence is complicated and, probably, unnecessary since environmental parameters are not as "easily" modified as pollutants.

The present preliminary survey was designed to scan readily available invertebrates from five estuarine systems in the southern Gulf of St. Lawrence for any pathological symptoms which could be attributed to pulp and paper industrial effluent. Three of the five estuaries contained effluent input. The numbers and frequency of sampling were limited but considered appropriate in an effort to detect obvious (as opposed to subtle) alternatives to the previous adult fish survey in species which hitherto have not been used in such investigations.

Materials and Methods

In May, 1996, five estuaries (Restigouche, Miramichi, Kouchibouguac (New Brunswick), Pictou and Margaree (Nova Scotia)).were sampled for both invertebrates as well as other multidisciplinary samples. Kouchibouguac and Margaree estuaries have no pulp or paper mill industries on them and were used as negative controls.

The invertebrate species collected initially included sand shrimp (*Crangon septemspinosa*), rock crab (*Cancer irroratus*) and blue mussels (*Mytilus edulis*). Two samples were collected, one in May and the second in September, 1996. Rock crab were dropped from the survey following May collections due to their inconsistent availability in all but one estuary. The remaining two species represented sessile primary feeders (mussels) and relatively non-migratory (compared with estuarine finfish sampled previously), zooplankton feeders (sand shrimp). Sample numbers consisted of 30 individuals per species per estuary (with the exception of shrimp collected in May from the Miramichi: n = 21). See Table 1.

	Shrimp			Mussel		
	May	Sept.	Total	May	Sept.	Total
Margaree	30	30	60	-	-	-

Pictou	30	30	60	30	30	60
Kouchibouguac	30	30	60	30	30	60
Miramichi	21	30	60	-	-	-
Restigouche	30	30	60	30	30	60
			291			180

Table 12.1. Sample collections and schedule for invertebrate pathology examinations - 1996.

All specimens were screened for grossly visible lesions, before being weighed, measured and processed for histopathological examination of the soft-tissues.

Gross observations were aimed towards cuticular lesions, exoskeletal (including gill and appendages) deformities and abnormal epibiont (fouling organisms) accumulation in shrimp. Mussels were screened for shell deformities, excessive fouling or perforation by shell-boring sponges and polychaetes and shell erosion.

Histology was chosen for its non-specific screening capacity and provision of a permanent record of specimen tissues. Tissues remaining after sectioning for slide staining were stored for additional histochemical processing or back-up sections, as required. Sections were collected from the mussels as outlined in Howard and Smith (1983) and the shrimp were sectioned as outlined for penaeid shrimp in Bell and Lightner (1988). Routine fixatives (Davidson's and 1G4F) and histological stains (Harris' Haemotoxylin and Eosin) were used for the preliminary examinations.

Soft-tissues were examined for necrosis (cell-death), neoplasia, saprobiont accumulation, abiotic and biotic foreign bodies (non-parasitic/proliferative tissue irritants), microbial and eukaryotic parasites, epithelial vacuolisation, oedema and haemocyte infiltration (systemic or focal = "granulomas"). Abscess lesions (lesions containing necrotic tissue debris), epithelial erosion, metaplasia (change in epithelial cell-shape) and hyperplasia (focal epithelial cell proliferation) were also noted. Signs of arrested or regressive maturation were also screened for in both species.

To date (February 24th, 1997) all specimens collected in May have been processed and examined. The list of preliminary observations from the September samples is also completed, however, prevalence and intensity data are still being compiled. These will be completed by the end of the contract and written up in a report format. The present report, therefore, constitutes a "near-complete" documentation of preliminary results.

Results

Shrimp (*Crangon septemspinosa*)

Several parasites and pathologies were noted in the specimens collected. None appear to be of direct disease significance, being detected in apparently healthy individuals transported to the laboratory and processed within 24 hours of collection. All specimens were fixed live and no processing artifacts were observed.

At least two types of intranuclear digestive tubule epithelial inclusion body were observed; four species of gill ciliate (Protista, Ciliophora) and one species of digenean metacercarial cyst. See Table 2 for May prevalence data.

	Margaree	Pictou	Kouchibouguac	Miramichi	Restigouche
Metacercarial cysts	3.3	6.7	30.0	9.5	16.7
Gill ciliates (total)	3.3	6.7	0.0	52.4	93.3
Hypertrophic	0.0	0.0	0.0	33.3	40.0

nuclear inclusions					
Sex Ratio (F:M)	25:5	28:2	29:1	19:2	30:0

Table 12.2. Prevalence (% of sample infected) data for histopathology of sand shrimp collected in May 1996.

Blue Mussels (*Mytilus edulis*)

Results from blue mussels were equally inconclusive with respect to overall mussel health. No significant pathogens were detected and no significant differences between estuaries were observed. Given the known sequestering capabilities of mussels for certain chemical pollutants, however, the selection of sampling sites may have been too distant from the putative pollutant hot-spots (Cheng 1993). See Table 3.

	Pictou	Kouchibouguac	Restigouche
Ancistrum mytili gill ciliates	63.3	45.0	43.3
Sphenophrya-like gill ciliates	6.7	3.4	3.3
Granulomas (focal granulocyte lesions)	3.3	0.0	16.7
Sex Ration (F:M)	18:2	14:15	15:15

Table 12.3. Prevalence data for histopathology of blue mussels sampled in May 1996.

Conclusions

None of the pathogens or pathologies observed to date give a clear effluent correlation. The reasons for this are manifold, but do not warrent exlcusion of invertebrates from environmental monitoring. Their use has been well documented for other studies and merits consideration for their continued inclusion in such elluent surveys (Galtsoff 1964; Gilfillan *et al.* 1977; Goldberg 1980; Sparks 1985; Sindermann 1990; Cheng 1993; Gardener 1993; Lightener 1993; Sindermann 1993).

Future Investigation Suggestion

One of the principal limitations to the preliminary survey was the lack of a negative control, i.e., establishing pathological results from known concentrations of pulp mill effluent. This is an imperative next step to establishing both lethal and sub-lethal influences on the lower trophic levels of estuaries under investigation. With this base-line data, subsequent surveys can distinguish with clarity between influences from inorganic compounds and those from normal physiological responses to estuarine fluxes (temperature, salinity, turbidity, etc.). This will provide a base-reference for histopahtology surveys or support services to growth and developmental surveys, e.g., caged mussels.

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Questions, Answers and Comments

Q. Were the sand shrimp sampled at the same times and places as the fish in Anne-Margaret Mackinnon's study?

A. Yes.

Q. What is known of the biology of sand shrimp?

A. Our species of sand shrimp lives 2-3 years, we don't know specific moulting times, and little is known of their general biology such as number of eggs per female.

C. Some work was done on *Crangon septemspinosus* in the Bay of Fundy by Sue Corey. Basic biological studies are planned for the southern Gulf of St. Lawrence starting in 1997. Much work has been done on the brown shrimp, *Crangon crangon*, which is commercially fished in Europe.

C. The reasons that we think that sand shrimp may be a good indicator species are that they:

1) are extremely abundant and easily sampled in the estuarine and coastal marine environments; 2) are a major component in the estuarine and marine coastal food web, being the major prey item for many juvenile and adult fish (and probably birds) in these environments (e.g., striped bass, tomcod); 3) have been shown to accumulate contaminants to different levels in industrialised and undeveloped rivers; and 4) have been used extensively in laboratory toxicology studies.

13. CAGED EXPOSURES, LIFE CYCLE AND BEHAVIORAL STUDIES WITH FISH...EXAMPLES AND COMMENTS.

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The use of cages to assess the effects of sediment exposure on Arctic grayling (*Thymallus arcticus*), and pulp mill effluent on Pacific salmon are presented to exemplify the use of such techniques in impact assessments. Similarly the results of a life-cycle study with pink salmon (*Oncorhynchus gorbuscha*) exposed to the water soluble fraction of crude oil are discussed. The use of a "mesocosm" approach, allied to specific assays is commented on in relation to the exposure of juvenile chinook salmon (*O. tshawytscha*) to an anti-sapstain chemical and pulp mill effluent. These comments are offered in relation to potential use within the EEM program, and accordingly positive and negative aspects of the techniques will be presented.

Arctic grayling held within 50L net cages in placer gold-mined (sediment-laden) and unmined streams in the Yukon survived for 96h in the latter situation: mortalities occurred in the former. However, an increase in gill parasites and serum glucose levels in the caged fish populations in both locations, relative to non-captive fish, indicated that the caged fish were stressed by the confinement. Other studies, that assessed the feeding of Arctic grayling in streams that were subjected to placer mining found that confinement reduced the feeding success of the fish relative to those fish captured from the respective creeks (feeding success was impaired in the placer mined stream; a function of visual impairment and prey availability). The same cages were used to assess the survival of juvenile Pacific salmon adjacent to the effluent discharge from coastal pulp mills in B.C. In these experiments the survival of fish was related to exposure to the mill's effluent and related variables. Confinement of juvenile salmon did not affect short-term (4-12d) survival at reference sites. These cages have value for the short-term holding of juvenile fish in low velocity waters.

Cages which enclosed a 6 x 0.5 x 0.5m volume of surface waters were employed adjacent to effluent discharges from pulp mills and at reference areas in coastal locations. The cages were used to hold juvenile salmon and to examine their volitional vertical distribution. Distribution shifts occurred in relation to effluent and variation in natural factors. The technique proved valuable in revealing behavioral changes, and the use (lack of long-term avoidance) of toxic waters by juvenile salmon despite the potential of such conditions to jeopardize survival. Behavioral adaptive responses to occupy surface waters were considered to override short-term avoidance responses to toxic conditions in these waters, resulting in the death of some fish. Laboratory studies with a Water Column Simulator confirmed the behavioral traits of juvenile Pacific salmon to occupy surface waters and their response to environmental changes. The cages have been validated as a technique to investigate the vertical distribution/behavior of juvenile fish within 6m of the water surface. They are particularly useful in waters that are vertically stratified.

The effect of oil on the survival of juvenile pink salmon to adult was assessed by exposing the juveniles to the water soluble fraction of crude oil for 10d prior to their release to the Pacific Ocean. The subsequent return of fish that had received a low (50ppb), or high (350ppb) dose of the water soluble fraction, or no aromatic hydrocarbons was determined from catches by the fisheries and in their natal stream. A total of approximately 270,000 fish were released (90,000 per year, 30,000 per treatment) with half-length coded wire tags. Despite evidence of some dose-dependent histological changes, and behavioral responses, there was no significant difference among treatment groups in the numbers of fish that were captured as adults. The relevance of this type of study to the EEM program will be discussed. Life-cycle studies of this type rely on population success as an end point thereby integrating the effects of "natural" stressors in addition to those of the chosen contaminant.

The exposure of juvenile salmon to pulp mill effluent at an on-site laboratory provided a controlled situation relative to an in-situ field investigation. Assessments of effects in relation to effluent concentration were quantified. An investigation of the potential for juvenile salmon to be exposed to the effluent was undertaken through an in-situ mark-recovery program. A predator-prey experiment revealed how exposure to sublethal concentrations of the pulp mill effluent rendered the exposed salmon more susceptible to predation. A similar susceptibility response has been determined after exposing juvenile salmon to sublethal concentrations of a lumber anti-sapstain chemical (TCMTB). The relevance of this test to the EEM program will be discussed. In complex effluent receiving waters the isolation of effects from one source is fraught with problems. By subjecting test organisms to relevant exposures to contaminants (concentration and duration) at an on-site facility using, for example, fresh effluent from one source, some of the complicating variables in the field location are removed. The subsequent choice of tests to indicate effects should be tiered to facilitate correlation's that will permit the consequences to organism survival, health and performance to be assessed. The predator-prey assay we utilized is one such test that has direct relevance to survival.

Questions, Answers and Comments

Q. In your life cycle study with pink salmon exposed to oil in early life, do you think that other sources of mortality masked any effects of the oil on return rates from the ocean?

A. Yes. We saw effects of oil exposure on the juvenile salmon but not the rates of adults returning to spawn. You must remember that fishing pressure is enormous and non-selective, unlike natural factors such as predation.

Q. How would you use this life cycle approach for the pulp mill EEM?

A. We still see salmon as a good indicator species for the west coast. We could use the mesocosm approach plus some field work to track disease etc. The difficulty in doing early life exposures is in holding so many fish without stressing them too much. We must assess the relevance of lab work too, we should use realistic parameters (e.g., temperature) and more than one species of fish.

14. THE MUMMICHOG, *FUNDULUS HETEROCLITUS*, AS A SENTINEL SPECIES FOR PULP AND PAPER MILL EEM SURVEYS IN THE ATLANTIC COASTAL ENVIRONMENT

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The Adult Fish Survey component of the EEM program for Canadian pulp and paper mills encountered two major problems in Atlantic Canada: availability and mobility of species chosen as sentinels (Parker 1997). Of 15 pulp and paper mills which discharged effluent into estuaries or the coastal environment, not one met its fish requirements in Cycle 1 (i.e., 20 males and females of each of 2 species in both nearfield and reference sites). Furthermore, the species selected as sentinels by these mills tended to be migratory. The most common sentinel species chosen were the winter flounder (*Pleuronectes americanus*) and the Atlantic tomcod (*Microgadus tomcod*), selected by eight and seven mills, respectively. Within the southern Gulf of St. Lawrence, winter flounder move tens to hundreds of kilometers between nearshore, summer feeding grounds and overwintering grounds in estuaries (Hanson and Courtenay 1996). Similarly, Atlantic tomcod make seasonal migrations of over 80 km between nearshore, summer feeding grounds and winter spawning sites in freshwater (Klauda et al. 1988; Courtenay et al. 1995). The extent of movement within seasons is less well known but is on the order of kilometers for winter flounder (D. Willis, Bedford Institute of Oceanography, pers. comm.) and is probably similar for tomcod. Furthermore, large fish like tomcod and flounder do not need to migrate actively to be displaced within estuaries. The fact that they live in the deep channel of the estuary means that they are exposed to the full extent of tidal movements of water. Tides can move water in the Miramichi Estuary, for example, 10 km in 6 hours. To put these movements in perspective, the reference stations for the Fish Survey carried out by a paper mill on the Miramichi Estuary were approximately 5 km upstream and downstream from the mill. A tomcod at the downstream reference station might easily be carried to the mill in a single tide. Species such as these are good biosentinels for the health of whole estuaries or sections of the coastal environment but not for specific point sources, such as pulp mill effluents, which may be diluted to less than 1% within 50 m of the discharge point. For the detection of impacts occurring on such a small geographic scale, highly sedentary species are required.

The mummichog, *Fundulus heteroclitus*, is both easy to catch in large numbers, and sedentary. Generally less than 10 cm in length, the mummichog is found in estuaries and tide pools all along the east coast of North America between southern Newfoundland and northern Florida. Within this range it is extremely abundant and easy to catch. It is a very plastic species, tolerating a wide range of salinities, temperatures, and oxygen concentrations. These characteristics have led to its wide use as fishing bait and for laboratory studies including many toxicological studies (e.g., Prince and Cooper 1995a,b). Spawning occurs in shallow waters between April and August and fish overwinter in holes of tidal streams or in the mud. A study of mummichogs in one stream showed that they moved less than 2 km upstream to overwinter in lower salinity water (Fritz et al. 1975). Mummichogs can be aged by otoliths and live to a maximum of 5 years. They eat sediment based prey including small crustaceans, polychaetes and insects, and are in turn preyed upon by fish such as striped bass (*Morone saxatilis*), bluefish (*Pomatomus saltatrix*) and birds such as herons (*Ardea herodius*) and kingfishers (*Megaceryle alcyon*). Given its numbers, the mummichog is an important part of the Atlantic coastal food web. Most importantly though, it is extremely sedentary during the spawning season, typically moving less than 36 meters along the intertidal shoreline of a creek or river (Lotrich 1975). Because of this sedentary nature, mummichogs are good biosentinels of small scale environmental disturbances. Iannuzzi and Bonnevie (1994) reported an extremely strong correlation between PCB concentrations in sediments and in mummichogs along a 1 km gradient from a PCB discharge site in a small river flowing into Chesapeake Bay.

Between 1992 and 1996, one of us (Couillard) studied the effects of bleached kraft mill effluent on mummichogs living in the Miramichi Estuary. Three populations were examined, living 4 km, 21 km and 39 km respectively downriver from a pulp and paper mill. To control for differences other than BKME along this gradient (e.g., salinity), two populations living in the neighbouring Bouctouche Estuary were also examined. Exposure to contaminants was measured both by direct chemical analyses and also through mixed function oxidase levels (MFO). Health was measured through: fin erosion, radiographic vertebral lesions, parasites, steroids in blood and production in gonads, histology, various blood measures including: hematocrit, concentrations of total proteins, glucose, bilirubin and sorbitol dehydrogenase activity, immunotoxicity measured by macrophage activity from the posterior kidney, genetic variability assessed through electrophoretic examination of hepatic enzymes, and developmental homeostasis assessed through meristic and morphometric counts. In addition to measurement of steroids, reproduction was examined through: temporal variation in gonado-somatic index (GSI), percentage mature females, fecundity of females, egg size, and embryonic malformations.

Mummichogs living nearest the mill showed a 2.5 fold EROD induction over populations living downriver or in the Bouctouche Estuary. By comparison, previous investigations with the more mobile tomcod and flounder had detected no such gradient in MFO activity throughout Miramichi Estuary (Addison et al. 1991; Courtenay et al. 1995). Preliminary chemical analyses of mummichogs revealed levels of PCBs and organochlorine pesticides up to ten times higher at the mill site compared to downstream, dioxins and chlorophenols typical of BKME were 30 - 40 times higher, and pentachlorophenol was 200 times higher. Fin erosion and vertebral deformities, effects that have been previously associated with exposure to BKME (but are not specific), were seen in the mill population (Couillard and Légaré 1994) as were effects on parasite load, hematocrit, blood proteins and glucose. The mill population also showed differences from other populations in number of fin rays and incidence of bilateral asymmetry in pelvic fin ray count. Reproduction was also affected by mill effluent (Leblanc et al. 1997). Gonadal maturation was delayed and egg size was reduced at the mill site. However, once reproduction was underway at the mill site, GSI and fecundity of mature females were greater than elsewhere such that despite delayed gonadal maturation, there was an overall increase in reproductive effort in mummichogs exposed to BKME. Interestingly, this did not result in a decrease in condition factor relative to other sites, suggesting that nutrients were abundant at the mill site.

In summary, the mummichog appears to be an excellent biosentinel species for the Fish Survey component of the EEM program for pulp and paper mills. It is abundant, sedentary and appears to show effects of exposure to BKME on health and reproduction. It should be noted however that even the relatively ubiquitous mummichog will not always be found within the 1% effluent plume of estuarine and coastal mills. In Miramichi Estuary, the nearest population to the mill is located at the nearest marsh, 3-4 km downriver from the 1% effluent plume. It is therefore impossible to establish cause-effect relationships with BKME because of the presence of other sources of contamination close to the mill such as sewage and historic deposits from a wood preservation plant (Courtenay et al. 1995). Where mummichogs are not available, other small, forage fish such as sticklebacks may serve as sentinels. Sticklebacks (Gasterosteidae) have the advantages of being circumpolar in distribution and relatively well studied. However, the four species of sticklebacks found in Maritime estuaries are smaller than mummichogs and are probably more mobile. In fact, we know of no other Atlantic, estuarine or coastal, fish species as well suited to the pulp mill Fish Survey as the mummichog. A recent consideration of fish species suited to field monitoring of exposure and effects of environemental contaminants also concluded that the mummichog was perhaps the best sentinel species (Myers 1996). Finally, in choosing sentinel species and sampling strategies, a thorough knowledge of the species, and population, biology is essential (e.g., Leblanc and Couillard 1995). For example, preliminary work with mummichogs in Miramichi Estuary indicated that there was a higher rate of malformations in embryos from females living near the mill than elsewhere (Couillard et al. 1995). Subsequent work revealed that malformations were more common at certain points in the spawning cycle than at others. When differences between populations in the timing of the spawning were taken into consideration, very little difference was seen in the rate of embryonic malformation. This example highlights the danger of comparing fish between sites in a single snapshot versus examining the entire reproductive cycle.

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Questions, Answers and Comments

Q. Were there any differences in the age structures of mummichogs at different sites?

A. I'm not sure. However, ages were recorded and taken into consideration in other analyses.

Q. How close was the "mill population" of mummichogs to the actual effluent outfall from Miramichi Pulp and Paper Inc.'s bleached kraft mill?

A. The "mill population" was located at Strawberry Marsh, approximately 4 km downstream from the outfall. It is interesting to note that, at least according to plume delineations mapped by Beak Consultants in the early 1990s, the 1% plume is very small on a falling tide and never comes anywhere near Strawberry Marsh. Nevertheless, effects which are consistent with exposure to BKME are seen in mummichogs at that site. That said, it must also be noted that Strawberry Marsh is the site of a former wood treatment operation (Domtar) and despite a comprehensive cleanup, conceivably some contaminants remain. Sewage may also be found in the area. Future work at this site will be complicated by the recent construction of a new bridge and a new sewage treatment plant at Strawberry Marsh.

15. USE OF FORAGE FISH AS SENTINEL SPECIES FOR EEM MONITORING

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Small fish species were evaluated for responses to pulp mill effluent, and these studies demonstrated that these species were showing responses to pulp mill effluent. We have used spoonhead sculpin (*Cottus ricei*), lake chub (*Couesius plumbeus*), and trout-perch (*Percopsis omiscomaycus*) (Gibbons, W.N. 1997. Suitability of small fish species for monitoring the effects of pulp mill effluent on fish populations. Ph.D. Thesis, University of Waterloo), and peamouth chub (*Mylocheilus caurinus*), and have new studies which will attempt to use log perch (*Percina caprodes*) and johnny darters (*Etheostoma nigro*) for comparison of responses with larger species.

Spoonhead sculpin exposed to kraft mill effluent in the Athabasca River demonstrated major differences in growth rate within 0.5 km of a pulp mill discharge, and significant differences were seen between exposed and unexposed sides of the river. Spoonhead sculpin demonstrated an overall increase in body and organ size, reproductive commitment, MFO induction and steroid hormone production relative to unexposed fish. Sculpin also showed a graded response with distance, and MFO induction persisted for at least 50 km downstream.

Further studies were conducted on the Moose River system to compare responses between small fish species and larger species collected simultaneously. Effort consisted of a comparison of trout-perch and white sucker whole organism and physiological responses downstream of a TMP mill. Initial evaluation of the data demonstrated differences in the responses of the two species. Trout-perch showed decreased length, weight, size-at-age and gonad size, while white sucker demonstrated increased weight, length, condition factor and size-at-age. A fish interpretation framework was modified and use of the information on age structure, energy utilization and energy storage suggested that the trout-perch and white sucker were responding to similar stressors.

It is obvious that forage fish species offer information suitable for effects monitoring.

Advantages

1. Small species represent the forage base and are more numerous than larger species.
2. Capture efficiency can be very good; it is possible to get all fish at a site in a short time period. These fish (most species) also survive capture better, especially since many of the capture techniques are less harmful (ie. Seining, electroshock).
3. Assumption is that mobility is much less than larger species.
4. There are not subjected to fishing pressure.

Concerns

1. Virtually nothing is known about the biology of most of these species (spawning time, fecundity, reproductive life history, growth rates, ages, etc.); background data on basic biology is needed for interpretation.
2. Samples size requirements are larger than estimated for large species, and due to the short life span, immature individuals are required to look at growth rates, etc.. We have been trying to get 35 males and females, as well as immature fish.
3. Reference sites become more difficult to standardize, and more care is needed. There are significant differences between rivers.
4. The responses can be different than those seen with larger species due to a variety of factors including different habitat utilization, shorter life span, different dietary requirements.
5. Reproductive outputs can be difficult to estimate in species which are fractional or multiple spawners.

Questions, Answers and Comments

Q. With regard to the lack of adults trout perch found downstream (i.e., fish downstream of effluent were smaller and younger than fish upstream), has anyone looked at predation?

A. We're trying to secure funding to follow up with community responses such as food web structure, energy flows etc. Also, in recent years mills have been modernized so we would like to see what effects changes have had on fish in the receiving environment.

Q. Trout perch live up to 5 years in the Athabasca River. Also, adults and juveniles occupy different habitats, could that explain the difference in age structure between your upstream and downstream sites?

A. We are aware of these facts, but we sampled as thoroughly as we could in both sites and caught adults upstream so we do not think that the lack of adults downstream was a sampling artifact.

C. We should look at similar endpoints in caged bivalves. Using several approaches together would make a much more convincing case than looking only at 1 species.

A. Agreed, a weight of evidence approach would be helpful.

Q. Spot-tail shiners have been looked at in the St. Lawrence; do you think they'd work in EEMs for the Great Lakes?

A. Any species should work. The limiting factor is lack of knowledge of the biology of the fish.

C. The problem is in finding good reference sites and sampling times. Can we really expect consultants to do this?

C. Researchers can optimize collections by better using whatever human resources are available and especially people with knowledge and familiarity with local fish populations. Remember that consultants generally lack such specific knowledge.

C. One problem for consultants catching forage fish is not necessarily lack of knowledge about fish; there is a learning curve to catching new fish at a new site. A good source for advice is anyone who has collected for museums. They have all kinds of different methods and knowledge.

Appendix A: Discussion Session #1
Monday, 17 February 1997

DISCUSSION, SESSION #1, Monday, 17 February 1997
(speakers are identified by first and last initial - see APPENDIX D, List of Attendees)

CH: What are you looking for exactly, with alternatives to the adult fish survey (AFS) - replacing finfish or looking for something completely different? What are the goals and objectives of the EEM program - to detect effects on the environment, or on finfish, or on invertebrates?

SC: The objective of the existing EEM is to determine whether the existing pulp and paper environmental regulations (PPER) are protecting fish, fish habitat and the usability of the resource.

KM: The EEM seeks to determine whether mills are in compliance with regulations, and for the AFS, whether they are affecting fish. How do we define "protect"?

SC: The adverse effects being assessed by the AFS pertain to survival, growth, and reproduction.

RA: Do any of the alternatives discussed so far provide surrogates to answer those questions? Can we answer them from, say, measurements of Scope For Growth (SFG)?

KM: I'm not sure that's the purpose. Where the AFS was successfully carried out in Cycle 1 and found no effects on fish, we can conclude that the PPER are working. We're looking for alternatives to use at sites where the fish survey used in Cycle 1 isn't suitable to pick up effects; not necessarily on fish alone, but for the site.

RA: Then isn't the benthic study alone enough to determine that? Do we really need an AFS as well?

SC: Aren't there cases where the benthic survey wouldn't pick up changes but fish would?

KM: Yes, there are differences in the response mechanisms; fish respond to benthos and to water chemistry, benthic animals don't necessarily do that.

MD: The benthic response is often one of nutrient enrichment, as was found in the Athabasca River.

CH: If we carry out surveys on invertebrates, at some point we'll have to link results to fish populations won't we?

KM: The purpose of the program was to define how widespread effects were, are effects site-specific or common to many mills? This could lead to site-specific regulations or changes to the overall PPER

RA: There are two different questions: are we looking for measurements to detect general environmental changes, or do we want to see evidence of various kinds of disruption in health manifested in growth, survival and reproduction of finfish? Sometimes these are parallel but sometimes not. I don't think we can answer them both with one question.

KM: For some of the issues we have to deal with, there is no kind of fish survey that could be conducted (e.g. trapped plume), and we have to come up with alternatives for those as well as for other situations where a fish survey is possible.

RA: Are there surrogates that can be predictive of effects on fish?

SC: Juvenile fish for instance, are there effects on juvenile fish where mills are located near nursery areas (e.g., Miramichi Pulp and Paper Inc.)?

AC: We must be able to separate out confounding factors, perhaps by focussing more effort on a bioassay or something like that.

KM: If we find that 10% of mills cause effects and all those mills have confounding factors, how do we deal with the situation?

AC: Exactly, so how do we get those others dischargers involved so they are monitoring the impacts of their effluents? Do we need assessment tools for municipal sewage for example?

SC: I think that we do. Ultimately we are probably heading towards a tiered approach in which we monitor the health of watersheds or basins and then use different tools to apportion effects to particular sources of contaminants. We should keep this model in mind as we make our recommendations for AFS so that monitoring for pulp mills fit into the larger picture later on. Peter, how does this fit into what you've seen in the oil industry?

PC: All the same thing, trying to figure out what differences mean But we are working in a more pristine environment on the Atlantic continental shelf than your situation in the coastal environment.

SC: Are people comfortable with caging studies?

KM: Is there a problem with bringing caged animals into areas where they're not normally found?

MS: It can be a concern; hopefully any permitting systems should take care of this. It is a bigger issue in fresh water than in marine waters.

MP: In the EEM program, the approach taken was to use different approaches: lab toxicity testing on individuals, and field components (population survey of fish, community survey of benthos). When looking at alternatives to the AFS, we need to keep the aspects of the survey that make it distinct from the other surveys. Maybe that'll help up to evaluate alternatives.

MS: The "TRIAD" approach is included in the *In situ* mussel approach. I think there should be more empasis on tissue residues because that's where I think regulations is going. You have bioassays - single species, semi-controlled experiments. You seem to be going toward site-specific because all so different; one size doesn't fit all. You may need different combinations of approaches on a site-specific basis.

MP: Okay, but what elements of the FS do we want to preserve?

PW: Given the problems of sampling fish, how do obtain the most information from the measurements on a few fish? What is your minimum set of health indicator measurements to make this assessment?

SC: Maybe the site could dictate the study. If it is a spawning area, do a spawning evaluation; if it is a nursery area, focus the evaluation on juveniles.

KM: The final structure of our decision tree will mean that if a mill wants to do somethig different from the standard program, they'll be forced to justify it but the program will be able to accommodate alternatives.

RA: Remember how much basic biological information is missing from species we use as sentinels - things like food preferences, migratory habits, breeding information.

AC: On the west coast we considered this for Cycle 1; DFO scientists provided advice to help mills and consultants select suitable sentinel species. But let's be realistic about what can actually be monitored. Effluent doesn't behave nicely so showing exposure is not easy.

PW: Chemical residues in bile are good indicators of exposure.

RA: We simply don't have enough basic biological information to interpret field data and conclude that fish are healthy or unhealthy so I say stick with laboratory tests.

SC: Is our problem that we haven't seen dramatic effects on fish in Cycle 1? Are fish living near mills all healthy, or at least the fish left to be sampled? For context, in the Hudson River, NY, the incidence of hepatic carcinomas in post-yearling tomcod has reached 90% in some years and fish do not live beyond 2 years compared to 8 in the St. Lawrence River. The problem there is obvious.

IB: We showed that exposure compromises migratory performance of adult salmon, that they'll go into contaminated areas and die there. So showing presence of contaminants in the environment is not enough. There are survival aspects that should be considered as well: predator escapement, swimming performance, behaviour, disease resistance, etc.

KM: The EEM program was supposed to identify research issues and government would do the research. The problem is that no moneys were set aside to address these issues. Research has now been left out of the program. What's the minimum acceptable to conclude that there is no problem? That's our question now.

IB: And who decides that acceptability?

KM: A small group of people who designed EEM. Their decisions are being questioned and the EWG is now supposed to figure that out.

IB: Growth and survival are important issues and there are bioassays to look at those.

SC: What about caged bivalves? If you show effects on growth, is that sufficient?

MP: I've some concerns about cage studies. Mussels caged in a trapped plume at depth is not realistic exposure - it would never happen in the real world.

MS: The point is that in a situation where you have no data, you can say nothing. It would be better to cage mussels than knowing nothing about the potential effects of that effluent. It would be a worst case scenario which can be made more realistic by looking at plume dispersion etc.

MP: It only makes sense to do cage studies if you know fish are exposed at some point, so if tracers have shown that they are exposed then a cage study might make sense.

KM: But if there was no exposure of fish to the effluent, either due to a lack of habitat, rapid dilution, no fish, or a trapped plume, the AFS would not be an issue.

IB: Fish can and will stay in bad environments and die there; we see it in the lab and field. For example, Pacific salmon key in on 12 degree C water during migration. Even if it is polluted and can be detrimental to their health, they will stay in the water layer, or area, that is this temperature.

MS: Exposure, behaviour, etc. are all important and we don't understand the interactions among all the different factors. If you cage animals in the plume, at least you get an idea of what the effluent could do. Maybe fish swim into it and die right away and that's why you don't capture live fish that show tracers.

CH: I agree; cage studies - in a trapped plume for example, could indicate that there is a potential danger of effluent and we need to see this.

PM: That is covered in the EEM program by bioassays, sublethal toxicity testing.

SC: There is a distinction between the laboratory toxicity tests and the fish survey; the former addresses the question: CAN effluent harm biota, while the fish survey addresses the question IS effluent harming fish or macro-invertebrates in the receiving environment?

AC: Caged mussels are an intermediate between lab tests and the AFS field study.

MS: If you see effects on mussels, then what?

SC: Yes, what if you carry out an AFS which shows no effects on fish but your caged bivalve study says there COULD be an effect if there were mussels there… then what?

PW: Salazar's approach has the advantage that it is linked to the chemicals.

MP: Yes, if you know what chemical to look for.

KM: The caged bivalve option will only be on one Alternative list for a certain kind of situation, it is not intended to be used in all situations. There are different reasons why AFS didn't work at various spots. Where a fish survey was successfully carried out in Cycle 1, or was sufficiently successful that with modifications it could be made successful, this would be the preference in Cycle 2. Ultimately, the decision about what kind of survey to do would be up to the mill and regional technical advisory committee. The fish survey EWG is working on a decision tree that might look something like this:

Alternative List A: Fish not available and environment not suitable for sampling
  - Caged bivalves
  - Mesocosms

Alternative List B: Fish not available but environment suitable for sampling
  - Wild bivalve survey
  - Wild crab/lobster survey

Alternative List C: Fish available but not in sufficient numbers
  - Work with small fish species
  - Increase sample size of a single sentinel species
  - Juvenile fish survey for growth and year class strength
  - Mesocosm studies

In judging the eligibility of alternatives for these lists, the following criteria will be applied. Alernatives should be:
  - Scientifically defensible
  - Cost-effective
  - Manageable in requirements and time frames
  - Capable of generating interpretable results
  - Capable of providing defined decision points
  - Available in the private sector
  - Relevant to the detection of adverse effects

Criteria of a suitable sentinel species are that they be abundant, exposed, and have easily-measured life-history variables, especially age and gonad weight. A hierarchy of species preference for Cycle 2 would be finfish-bivalve-prawn-crab/lobster. Decapods score lowest on this list because they cannot be aged and therefore cannot easily provide information on growth.

There is a similar program in Sweden - measurements on individuals, populations, tissues etc. Their approach is simpler because all of their mills have similar fish species and habitats. They use an annual program and have developed a better understanding of the biology of their sentinel species.

CH: What does "fish not available" mean?

KM: That in Cycle 1 no fish were caught.

KM: The EWG wants to clarify that people need to be open to alternatives. For example, if a consultant comes up with another species they want to use and they can justify it, then the regional technical advisory panel has to be able to listen to them

MS: The choice of alternative approaches should depend on whether fish are being affected by contaminants in the water colomn or benthos.

AC: Approaches will differ with the receiving environment too. For example, marine mills might select one finfish and one bivalve species.

MS: If we have background data, we could compare data in fish and bivalves and see if effects are happening together. If such data aren't available, I think that it should be research recommendation to collect synoptic measurements between fish and bivalves to see if there are relationships.

KM: Some synoptic fish/bivalve data do exist. Ian Smith of Ontario MOEE did caged bivalve studies for 2 years in Jackfish Bay, Lake Superior.

SC: We should discuss the rationale of the 3-year cycle approach.

AC: Is it better to do a simple survey annually or a more complex survey every 3 years? This question needs to be resolved at a higher level than the decision trees for the FS or other EEM components

BA: How will we improve measures of effort in cycle 2, when we couldn't compare among mills in cycle 1? Are we going to discuss standardizing measurement of CPUE (catch per unit effort)? Also, temperature/depth profiles should be included in all fishing programs.

KM: It is difficult to standardize effort unless we standardize the method used to fish; some people took 3 weeks, some 1 night. Effort will go down as people learn how to get fish more efficiently. I'm open to suggestions though.

BA: I just want to know how we can determine if fish are really there; just because the consultant didn't catch any doesn't mean there aren't any there. We need to evaluate effort in order to determine this.

Appendix B: Discussion Session #2
Tuesday, 18 February 1997

DISCUSSION, SESSION #2, Tuesday, 18 February 1997
(speakers are identified by first and last initial - see APPENDIX D, List of Attendees)

SC: Do we need a synthesis of studies which have examined Health Assessment Indices in fish exposed to pulp mill effluents across Canada?

BA: Many different workers across Canada seem to be using FHA - Stella Swanson for example. Maybe right now we need a literature search to bring togther all available knowledge.

SC: Those who really enjoy meetings might suggest a workshop.

AC: Sure, but it would be better to get a consultant to put together a review and circulate it first.

SM: ICES has done a review on FHA for North Sea fishes, this would be a good place to start. For the invertebrate side, we should look at experimental work to determine dose/effect relationships before instituting health assessments in field programs.

AC: That's why a workshop might be useful, you've got all these experienced mill-related sources, bring them all together. Much data and expertise will not have been published.

KM: I want to hear what people think about tumours, lesions etc. They are ugly but there's not a lot of evidence that they are that harmful to fish. If fish have lesions but are performing well with respect to growth, survival and reproduction, are the lesions a problem?

BA: Our data from the Columbia River show that despite a lower HAI (more lesions and tumours), downstream fish were bigger at age, had higher condition, higher GSI and LSI in some cases than fish upstream, so other than CDS, their health didn't seem to be compromised.

SC: MFO induction has frequently been shown to occur in response to exposure to pulp mill effluent as well, but again, we have not been able to tie this to effects on growth, reproduction and survival. Maybe we should be going right to the bottom line (growth, survival, reproduction); if we see changes there, then start looking at other things.

MS: Yes. We compared biomarkers to growth rates in bivalves living in San Diego Bay, and in no case were the biomarkers more sensitive than growth. I think you'll see the same with fish - growth will be the most sensitive in the end.

KM: Another way to ask the question is what is the minimum data set required to accept that there is no problem at the site? The decision for Cycle 1 was to evaluate gonad size, liver size, growth and age structure. If they were "normal", we would say that the fish population was okay. If we want to change that we'll have to justify it.

IB: No one has evaluated the performance of live fish before they are sacrificed, swimming performance for example. Fish with fin rot probably swim less well than healthy fish.

AC: In the Fraser River, there was no correlation between the HAI and histopathological changes.

KM: We found that goldfish near a steel mill recovered from very bad furunculosis lesions; that was not a big health problem for them.

SC: On the other hand, we and other workers have found a strong correlation between expression of atypical furunculosis and mortality in Atlantic tomcod.

AC: We should synthesize the HAI information collected during Cycle 1. If disease is a problem at a given site then look at it, but don't go screaming for it everywhere.

SC: Has there been enough information collected during Cycle 1?

MP: With HAI, it's very quick to measure and can end up being useful.

KM: Requiring it would also ensure that the consultant actually looks at the liver, gonads, etc.

BA: Right now, consultants have to do the internal exams and are killing the fish. We could provide a checklist to help them do a quick external screening. If problems are detected, they could proceed with a more detailed examination.

SM: There are multiple causes of gill and liver lesions too which shouldn't be lumped into one and the actual causes ignored.

AC: Results will be affected by the level of training workers have. We need standard methods for describing observations.

PW: Are we trying to prepare a guideline to lead to next phases?

BA: Part of the HAI is to look at bile colour but that doesn't mean much. People can go astray if they stick to those protocols too closely. What we basically need is some sort of screeing technique a the beginning.

MD: Yes, the HAI observations are subjective. Maybe we need a centrally coordinated effort with one group that leads the study and has a standardized program, trained people. They could look at the sites where screening in intital surveys has shown a problem.

SC: That's right, initial screening for lumps and bumps requires little training but histology seems to be very time-consuming and requires highly trained personnel.

CH: Consistency is still going to be a problem. We could generate a guidebook to standardize observations for consultants.

PM: I've used HAI at 2 sites and I understand that you are not supposed to interpret what you are seeing; just looking for differences from normal. Is the gill pale compared to your experience of normal? The index is not to say whether it is more severe or damaged or whatever, just is there a difference. If there is then you have a basis to go in and look more closely, get a pathologist to look at specific problems, etc.

SC: But would 2 or 3 people give the same result?

SM: There are guides available for HAI, and perhaps a workshop would be a simple way of settling on standards.

PM: Anyone with some experience will be able to do it easily. We shouldn't get too worried about what differences mean at the early stage.

MP: There is subjectivity but one thing we did is a simple scoring system for fat level. There was no problem, regardless of who was doing the work, we always saw that females had higher fat levels than males, as we should find.

SC: We should think about what alternative methods or supplements are available for Cycle 2? What methods need further refinement or testing and could possibly be used for Cycle 3? What methods still require further research before they could be used routinely in an EEM program?

KM: There are criteria listed in the EEM Requirements Document for the acceptability of tools. We should go through all the alternatives discussed here and look at them according to these criteria:

Scientifically defensible
Cost-effective_
Manageable requirements and time frames
Generate interpretable results
Provide defined decision points

Let's start with mesocosm studies. These could be used for benthics now. Would we allow someone to try to develop it for the FS during Cycle 2 at a site that can't be looked at in any other way? I think so.

RA: I have a general question regarding the fish survey. The experience in Cycle 1 was that in fresh water, better controls were available, more fish were caught, and better interpretation of data was made than in the marine situation which seems to be much more difficult. For the marine environment, does it make sense to keep trying field observational studies rather than laboratory experiments? All the money spent on observational studies that might lead to ambiguous information could be put into a generic experimental study like Ian Birtwell described this morning, resulting in much less ambiguous information. This approach would depart from the traditional monitoring but do we want science or do we want comfy monitoring? Is it better to spend money at each mill to do a cursory study or is it better to pool the money and conduct a few very comprehensive (broader based) studies?

SC: That approach would mean a 3-6 year holiday from fish monitoring.

RA: Not necessarily, we could get results in less than 2 years from Ian's study.

AC: Mills don't spend that much on field studies. Also we'd only be able to answer a couple of questions, or the questions at a couple of sites, but what about the rest? That's why it may be appropriate for some mills to do that but for others to keep the FS where it works. If an issue is identified at a site, then design a specific experiment which meets the program requirements.

KM: We can't force research. We can recommend it, but we can't force mills to put money into research.

RA: This is not a research question per se. We are asking, does mill effluent affect growth, survival or reproduction in fish, or not?

AC: The distinction between research and monitoring can be tricky. We can suggest a multi-mill, salmon life cycle study, put it down as an option, but I don't know if we can implement it. This is no different from the mesocosm approach in that sense.

KM [agrees]

IB: Trying to determine effects in the estuarine environment is fraught with so many problems, it's a waste of money, when no results are ever obtained.

SC: Do you think that Colin Levings (DFO, Pacific Region, Marine Environmental Science Division) would agree that the estuarine habitat is so complex that there's no way to answer these questions or perform monitoring there.?

IB: He has looked at pristine estuaries so far. Looking at estuaries with multiple pollutants is different.
SC: One concern that I have in moving from the field to the laboratory is that your question changes from DOES effluent impact biota in the receiving environment, to CAN effluent impact biota..

IB: You can at least get inferences from cage experiments.

MD: Experimental doesn't necessarily mean research either; it's an approach.

SC: Developing new techniques doesn't fit under a monitoring program though. I personally think that government and industry should be in the business of developing better monitoring tools but possibly not under this EEM program.

IB: But how can you tell what's happening in monitoring when you only ever sample live fish, that is, the survivors? You don't know how many fish died. That's what more controlled studies, such as mesocosms, show.

MS: A dye study doesn't necessarily show where contaminants from plume go. You need tissue residues to pair with responses of fish in exposed area. If you have similar conditions in a lab setting to compare to field observations, you can get a better understanding of the real pathways of contaminants in the environment. What do these dye studies mean anyway?

SC: Good point. The EWG has to take note of that; we have been defining the area impacted by effluent as being that within the 1% (or greater) plume. Effects extend beyond this area in some situations.

CH: Exposure can at least be quantified with respect to time and concentration if you use a controlled situation like a mesocosm.

KM: I don't think that we have time in this discussion to screen each potential alternative approach through the criteria listed above. This task should be left to the Expert Working Group.

PW: Any alternative approaches should link to the other study tools, benthic studies and laboratory toxicity tests for example.

SC: I learned just last week that the benthic survey component of the pulp mill EEM has experienced similar problems in the marine and estuarine environments. It is noteworthy that there has been virtually no integration at all of different components of EEM so far.

PW: It is not useful to polarize discussion into lab and field work. We will likely need both approaches, and integrate them for a proper risk management.

Appendix C. Workshop Program.

A Workshop to Discuss Alternatives to the Adult Fish Survey
for the Pulp & Paper EEM Program

Date: 17,18 February 1997

Location: Room 1524, Queen Square
Environment Canada Regional Office
45 Alderney Drive
Dartmouth, NS

Monday 17 February - Agenda:

0900: Welcome & Introductions

0910: Roy Parker & Simon Courtenay - Purpose of Workshop

0920 - 0940: Experiences with the first cycle EEM program Adult Fish Surveys in the Atlantic Region - Roy Parker

0940 - 1000: Experiences with the first cycle EEM program Adult Fish Surveys in the Pacific Region - Al Colodey

1000 - 1020: Coffee Break

1020 - 1040: Experiences with the first cycle EEM program Adult Fish Surveys in freshwater studies in Central Canada - Sue Humphrey

1040 - 1100: The use of bivalves and crustaceans as sentinel species for the EEM Adult Fish Survey - Chris Hawkins

1100 - 1200: Refined caged bivalve methodologies as part of environmental effects monitoring: characterizing exposure and effects - Mike Salazar

1200 - 1300: Lunch (provided)

1300 - 1330: A biomonitoring system using bivalves for assessing the spatial extent and temporal variations in environmental impacts from chronic pollution - Peter Cranford

1330 - 1410: Applications of mesocosm technology to assess environmental impacts from pulp mill effluents and other point sources - Joseph Culp and Monique Dubé

1410 - 1440: The application of the scope for growth approach to assess environmental impacts from pulp mill effluents and other point sources - Richard Addison

1440 - 1510: Status report from the Fish Survey Expert Working Group - Kelly Munkittrick

1510 - 1530: Coffee Break

1520 - 1630: Discussion period

Tuesday - 18 February 1997 - Agenda

0830 - 0850: Application of a fish Health Assessment Index approach for evaluating pulp and paper mill effluent impacts on fish in tributaries to the St. Lawrence River - Anne-Margaret MacKinnon

0850 - 0910: Use of a fish Health Assessment Index approach for evaluating pulp and paper mill effluent impacts on fish - a west coast perspective - Bonnie Antcliffe

0910 - 0930: Estuarine survey of sand shrimp and mussel pathology: 1996 results - Sharon McGladdery

0930 - 0950: Environmental monitoring studies using caged finfish: experiences, advantages, disadvantages - Ian Birtwell

0950 - 1010: Coffee break

1010 - 1030: The potential for utilizing small, estuarine forage fish as sentinel species for EEM studies - Simon Courtenay and Kelly Munkittrick

1030 - 1145: Discussion Period

1145 - 1200: Workshop Wrap-up - Simon Courtenay & Roy Parker

1300 - 1630: Fish Survey Expert Working Group Meeting (for EWG members only)

Appendix D. List of Attendees

Alternatives to the Adult Fish Survey Workshop - Attendees

Addison, Richard - DFO, Institute of Ocean Sciences, Sidney, BC
Antcliffe, Bonnie - DFO, Vancouver, BC
Birtwell, Ian - DFO, Vancouver, BC
Colodey, Al - EPB, North Vancouver, BC
Courtenay, Simon - DFO, Moncton, NB
Cranford, Peter - DFO, Bedford Institute of Oceanography, Dartmouth, NS
Culp, Joseph - NHRI, Saskatoon
Dube, Monique - Washburn, Gillis Associates, Fredericton, NB
Hawkins, Chris - Trident Environmental Consultants, Dartmouth, NS
Langlois, Claude - EPB, Montreal
Humphrey, Sue - EPB, Toronto
Luce, Shelley - contractor for AFS, EWG
MacKinnon, Anne-Margaret - DFO, Halifax, NS
Martel, Pierre - PAPRICAN, Montreal
McGladdery, Sharon - DFO, Gulf Fisheries Centre, Moncton, NB
Mills, Ken - DFO, Winnipeg
Munkittrick, Kelly - EC, NWRI
Paine, Mike - Paine Associates, Vancouver
Parker, Roy - EPB, Dartmouth
Rawn, Gary - DFO, Ottawa
Salazar, Mike - Applied Biomonitoring, Seattle
Spafford, Mark - ALPAC, AB
Tache, Michel - ECB, Ottawa
Wells, Peter - ECB, Dartmouth

Regrets

Lawrence, John - NWRI
Levings, Colin - DFO, Vancouver
Megraw, Stan - Domtar, Cornwall
Servos, Mark - EC, NWRI
Voss, Ron - PAPRICAN

Appendix E. Dungeness crab (*Cancer magister*) usefulness in Environmental Monitoring Studies

Editors Note: The following was not presented at the Dartmouth Workshop but was referred to by a number of participants during presentations and discussions. To support the recommendation that decapods not be used in future fish surveys because they cannot be aged, the following memo was kindly contributed by Dr. Glen Jamieson (DFO, Pacific Region).

Dungeness crab (*Cancer magister*) usefulness in Environmental Monitoring Studies

Glen Jamieson,
Pacific Biological Station, Nanaimo, BC
December 18/96

Crustaceans, and Dungeness crab (*Cancer magister*) in particular, have been proposed as a sentinel organism for the monitoring of marine environmental consequences of pollution in the marine environment. This is because large crustaceans are relatively high in the food chain, meaning pollution products may be concentrated in crab with consequently presumed greater liklihood that physiological effects will be manifested. Usage of crab has been suggested in two ways: 1) growth, or condition, consequences on individual crab, to be determined from population sampling and detection of general population trends (e.g., reduced growth, unusual population size structure, etc.) and 2) uptake and concentration of toxic compounds in particular organs or tissues.

There are major difficulties in relating possible discerned population effects to potential causes, i.e., pollution. Firstly, when crab moult, they shed all their hard structural parts, meaning that crab of any size cannot be accurately aged. Ages are inferred from looking at modes in size frequency data over a number of years and trying to relate them back to some point in time, representing larval settlement. Problems are that growth rate probably varies annually, dependent on water temperatures, food availability, competition, etc., and definitely seems to vary within individuals of a yearclass. These yearclass differences are compounded as the animals grow, so that a male crab yearclass around legal size has a mean size standard deviation of about 13 mm, meaning that 95% of the yearclass are between ± 2 SDs (140-190 mm) of 165 mm carapace width (CW; spine-to-spine). Growth in crustaceans is discontinuous, with sudden jumps in an individual's size when a crab moults. Growth is the result of two variables: size increment at moulting (fairly well described in the literature and reasonably constant as a percentage of pre-moult size) and frequency of moulting. The latter changes with the onset of sexual maturity, being fairly constant across both sexes before maturity. After maturity, female growth becomes quite complicated by reproduction, with considerable energy devoted to egg production and possible delays in moulting arising from external egg incubation. In males, it had previously been thought that because sperm production had a relatively low energy cost, moulting frequency was relatively predictable and probably annual. However, my research is suggesting that population size structure of mature males, affected by fishing removal of large males (maturity is reached at about 150 mm CW), may be affecting moulting patterns of sublegal, mature males. These smaller, presumably slower growing, sublegal males do most of the mating in the absence of dominant larger males, and most seem to never moult to a larger size later in their lives. For reasons unknown, but possibly related to either physiological consequences of mating or greater energy expenditure and/or loss of feeding opportunity while the males carry the females around in mating embraces, many, if not most, of these males seem to have at least a 2-year intermoult period, with increased shell disease and morbidity evident in their second intermoult year. Many of these crabs seem to die of what I tentatively call natural senescence. The consequence of all this is that unless a large sample size of the population is obtainable and collected, use of "population" characteristics in any meaningful cause/effect sense will be difficult.

A second problem, common to many species in addition to crab, is that the marine environment is not a homogeneous one, but rather consists of a number of discrete "habitats", many of which may not be immediately obvious to us as humans. The top and bottom sides of rocks often have different biological communities, for example, and the presence or absence of other animals (prey or predators) can make specific habitats desirable. Crab in the field are typically segregated by both size and sex as a result of specific habitat needs by different life stages, and this can complicate experimental design. One would normally not expect to find adult male and female crab together for extended periods, and field studies should acknowledge this. Prior to any study around a mill, a broad-brush survey should be undertaken to identify important preferred habitats, at least at that time of year, for the life stages of the species being investigated. Subsequent sampling should acknowledge the pattern determined and be designed with this in mind.

From a toxin uptake perspective, crustaceans are probably useful, as even large crabs such as Dungeness do not move too much and are often confined to specific substrate types. However, crab are often spatially separated by size and sex, so some understanding of spatial habitats preferred by different size crab may be important so that exposure periods can be estimated. General data are available, but some site-specific studies may be necessary at relevant locations.

Appendix F. Life cycle studies with salmon....Effects of pulp mill effluent.

Editor's Note: During discussions, considerable interest was generated by the suggestion that Pacific salmon life cycle studies be considered as an alternative to the fish survey for pulp and paper mill EEM. Drs. Birtwell and Addison (DFO, Pacific Region) were asked to prepare a summary proposal for such a study, the result of which follows.

LIFE CYCLE STUDIES WITH SALMON....EFFECTS OF PULP MILL EFFLUENT.

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Introduction.

Assessments of the effects of pulp mill effluents on economically-important Pacific salmon have most frequently been carried out on juveniles (individuals or groups) under laboratory conditions. Determining the effects on individuals under controlled conditions has much scientific merit, but the application of results to predict the population level response from such an indiscriminate pollutant stressor is extremely difficult, even when the experimental conditions closely mimic those in the wild: the deductions regarding effects on populations in the wild may be misleading. Similarly, attempts to determine effects in the field are severely limited by our inability to assess exposure to contaminants (concentration and duration), losses and debilitation of individuals (irrespective of cause), inefficient capture techniques etc. In addition, for example, chemical, biochemical, and histological assays on a (previously-living) portion of a population provide valuable insights into the potential harm to individuals from exposures to stressors, but provide little information related to performance and population success.

The assessment of effects can be enhanced substantially by combining a controlled exposure to the effluent with the natural challenges of life at sea. Thus both indiscriminate (pollutant exposure) and discriminate (e.g. predation) factors which affect population success may be integrated to provide greater relevance to the prediction of the effects of exposure to pulp mill effluent.

A life-cycle study may be used to determine the survival of populations of salmon exposed to pulp mill effluent at the sensitive, and relevant, juvenile stage. Such an approach has been successfully applied for the first time with respect to the effects of the water soluble fraction of crude oil on pink salmon survival to adult (Birtwell et al. 1996). The controlled exposure of populations of salmon juveniles to pollutants, followed by their release to the Pacific Ocean to complete their life cycle and return as adults, will permit an assessment of the effects at the population level. At the same time, a detailed analysis of effects on individuals can be made during the exposure period to relate to the numbers and health of returning adults.

The following comments are provided to indicate the nature of such a program as it may apply to an assessment of the effects of pulp mill effluent at the population level.

Species.

The choice should be guided by the logistics of the experiment, and the return of information in a timely fashion. Accordingly, species with a long life cycle would be a poorer choice than those with a shorter one. But, because the infrastructure is in place in the Pacific Region to determine the survival of tagged (CWT, adipose-clipped) individuals it would be possible to assess the effects on a longer-lived species such as chinook salmon. Irrespective of this concern, the experiment would need to be replicated. Pink salmon have the shortest life span, but because they are harvested in an indiscriminate manner, often in substantial numbers, and there is difficulty in providing the infrastructure to recover tagged fish from the fisheries, their utility can be problematic. In contrast chinook salmon could be used, and a good retrieval process is available from the fisheries, albeit over a protracted period because of their long life cycle.

Potential program.

Populations of juvenile salmon would be exposed to environmentally-relevant concentrations of pulp mill effluent for a realistic period of time (an industry-government decision) and control waters (salt or fresh water depending on species chosen, life cycle stage and experimental location).

Tagging.

Prior to the exposure the fish would be tagged (coded wire), and marked by clipping their adipose fin. Tag retention must be assessed prior to release to provide an estimate of the population of tagged fish.

Holding conditions.

The fish must be held in optimum conditions so that loading densities are not stressful. This requirement is a potential limiting factor, for the experiment will require the holding of thousands of fish. Depending on the species, life cycle stage chosen and known survival potential for the stock, holding facilities, and their supply of water and effluent, may be problematic. It is, therefore, necessary to calculate the expected return of control fish from release and determine the numbers of fish to be accommodated in the experiment. In studies with pink salmon fry, three batches of 30,000 individuals were held in separate troughs (control, and two treatment groups, respectively) for 28 days, in each of the three experimental years (Birtwell et al. 1996).

Location.

The location for the exposure must be close to a source of fish (hatchery) and fresh effluent, and have the potential for recovering the adult fish on their return to spawn. It would be possible to imprint the juvenile fish on non-natal stream waters (e.g. use of morpholine), if a source of fish was not close to a pulp mill.

There are few locations that meet all criteria, and the Port Mellon mill seems the most likely candidate, having a limited-length Rainy River adjacent to the outfall which discharges to the waters of Howe Sound. Hatcheries are close by, and the fish could be imprinted on home waters (assuming incubation and rearing opportunities) with or without the use of additional substances added to the waters.

Proximity to the estuary of the Rainy River, and the availability of fresh river water will permit an exposure of juvenile salmon to either fresh water or brackish-water conditions. The former would be more favorable: by using the brackish waters of the Rainey River estuary there would be the potential for dilute effluent to be pumped to the holding and exposure facilities due to mixing conditions and tidal effects in the proximal effluent receiving area.

The release of juvenile salmon exposed to dilute effluent in fresh water (rather than exposed to sea water when larger and older), over part of the time that they would be on their downstream migration, would permit smaller individuals to be used, and thereby reduce the need for extensive holding facilities (through a reduction in loading densities). The fish would continue their life in the ocean after passage through the estuary of the Rainy River and the waters of Howe Sound and into Georgia Straight and the Pacific Ocean.

Ancillary assessments.

During the time that the fish are held in the experimental facilities assessments would be made of their responses (biochemical, histological, physiological, behavioral, health, metabolism etc.), and the effluent to which they were exposed would be characterized.

By adding numerous assessments of the fish during the exposure period it may be possible to reveal the potential causes of mortality once the fish are challenged to life in the ocean. It would also be possible to correlate and assess, in a highly relevant manner, the utility of many of the tests that are currently used to assess sublethal exposure to pulp mill effluents, to survival under natural conditions.

Recovery of adults.

Through the "Head Recovery Program" in the Pacific Region, fish captured without an adipose fin should have their head removed and sent for tag retrieval and reading. Accordingly, fish captured in the fisheries and in their natal stream (alive and via dead pitch) would be assigned to the respective treatment they received as juveniles. In turn, an assessment would be made of the effects of treatment, and subsequent natural challenges on survival to adult. Analyses of the returning adults that were captured alive would be made to ascertain differences among treatment groups. The exact nature of analyses, aside from determinations of length and weight, gonad and liver, could be guided by the results of analyses done during the exposure period (e.g. histology, biochemistry) in addition to other valuable measures of health and performance.

Using the returning salmon for brood stock in subsequent experiments is desirable but probably not feasible considering the time frame over which one would need to wait before carrying out another experiment. It would be preferable to expose populations of juvenile salmon each year for at least three replicates. In this way the vagaries of "ocean climate" and other uncontrollable variables may be incorporated into the experiment. At the least, this would mean an experimental duration of four years with pink salmon as the test subjects to possibly eight or nine years if chinook salmon were used. Despite the duration of the work the benefits of environmental relevance outweigh this problem, and the study will provide the most meaningful information on the survival of populations of salmon, exposed to pulp mill effluent, to adulthood. (A compromise could be made on the approach outlined above, and replicate exposures could be undertaken in one year, thus reducing the overall costs but eliminating some variables and increasing the risk of failure due to unforeseen events).

Costs and relevance.

The costs of the replicate pink-salmon life cycle study which spanned six years (experimental and data analysis) was approximately $600,000. This did not include the provision of power to the on-site laboratory, the rearing of fish and the provision of holding troughs, nor the salaries of DFO staff involved with the project (estimate: $120,000).

When one considers the relevance of the experiment to the industry and regulators, aside from the scientific findings, the costs are relatively small. The potential of cost sharing among pulp mill companies should be assessed and perhaps such a program could be considered in lieu of some monitoring requirements under the EEM program. The relevance of the findings to other pulp mills using the same processes would be high. For other mills, the relevance would be lower, but it is likely that from the assessment of the effects of the effluent on juvenile salmon during the exposure period, that certain measured variables will correlate with adult survival and treatment. In this case it could be anticipated that the application of these tests to fish exposed to other effluents would yield good predictive results on population survival.

Reference.

Birtwell, I.K., R. Fink, R. Alexander, W. Bengeyfield, and C.D. McAllister. 1996. Survival of pink salmon (*Oncorhynchus gorbuscha*) to the water soluble fraction of North Slope crude oil. Can. Tech. Rep. Fish. Aquat. Sci. 2095: 49p.

Appendix G. Detailed Evaluation of Alternatives to the Fish Survey

Editor's Note: Tables that follow provide our own assessment of each alternative considered at the Dartmouth Workshop, ranked against the criteria most of which were suggested by Dr. Munkittrick (see Discussions), namely that a viable alternative should be:

Scientifically Defensible
Cost Effective
Flexible (available in the private sector)
Built on findings of research (relevant to the detection of adverse effects)
Manageable in requirements and time frames
Capable of generating interpretable results
Suitable for a weight of evidence approach
Capable of providing defined decision points

Proposed Alternative Method: Wild Bivalve Survey

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	•			
Cost Effective	•			
Flexible	•			
Built on Findings of Research	•			
Manageable	•			
Interpretable Results	•			
Suitable for a weight of evidence approach	•			
Defined decision points	•			

Proposed Alternative Method: Caged Bivalve Studies

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	•			
Cost Effective		•		
Flexible	•			
Built on Findings of research	•			
Manageable		•		
Interpretable Results	•			
Suitable for a weight of evidence approach	•			
Defined decision points	•			

Proposed Alternative Method: Habitrap

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	•			
Cost Effective		•		
Flexible		•		
Built on Findings of research	•			
Manageable		•		
Interpretable Results		•		
Suitable for a weight of evidence approach	•			
Defined decision points	•			

Proposed Alternative Method: Bivalve Scope for growth

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	•			
Cost Effective		•		
Flexible		•		
Built on Findings of research	•			
Manageable		•		
Interpretable Results		•		
Suitable for a weight of evidence approach	•			
Defined decision points		•		

Proposed Alternative Method: Mesocosm with Fish

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	•			
Cost Effective		•		
Flexible	•			
Built on Findings of research	•			
Manageable		•		

Interpretable Results	*			
Suitable for a weight of evidence approach	*			
Defined decision points	*			

Proposed Alternative Method: Fish Health Assessment Index (HAI)

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	*			
Cost Effective	*			
Flexible	*			
Built on Findings of research	*			
Manageable		*		
Interpretable Results		*		
Suitable for a weight of evidence approach	*			
Defined decision points		*		

Proposed Alternative Method: Invertebrate Pathology

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	*			
Cost Effective		*		
Flexible	*			
Built on Findings of research		*		
Manageable		*		
Interpretable Results		*		
Suitable for a weight of evidence approach	*			
Defined decision points		*		

Proposed Alternative Method: Caged Finfish Studies

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	*			
Cost Effective			*	

Flexible		•		
Built on Findings of research		•		
Manageable		•		
Interpretable Results		•		
Suitable for a weight of evidence approach	•			
Defined decision points		•		

Proposed Alternative Method: Fish surveys using small forage fish

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	•			
Cost Effective	•			
Flexible	•			
Built on Findings of research	•			
Manageable	•			
Interpretable Results	•			
Suitable for a weight of evidence approach	•			
Defined decision points	•			

Proposed Alternative Method: Salmon life cycle study

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible		•		
Cost Effective				•
Flexible				•
Built on Findings of research	•			
Manageable		•		
Interpretable Results				•
Suitable for a weight of evidence approach	•			
Defined decision points	•			

APPENDIX III

REGIONAL REVIEWS AND SUMMARIES

prepared by

S. Luce and K. Munkittrick

(not reviewed by the FS EWG)

February 1997.

CONTENTS

EEM Cycle One Fish Survey Reviews and Summaries:

Atlantic Region
Quebec Region
Ontario Region
Western and Northern Region
Pacific and Yukon Region

EEM CYCLE ONE FISH SURVEY REVIEW AND SUMMARY: ATLANTIC REGION

PART ONE: INTRODUCTION AND REGIONAL SUMMARY

Seventeen mills conducted Cycle One studies in the Atlantic region. Twelve mills discharge to marine/estuarine environments and five discharge to freshwater (river/lake) environments.

Determining catch success was considered a key element of Cycle One and catch success for the Atlantic region is presented in Table 1. Two freshwater mills and 12 marine/estuarine mills were successful for at least one species (*i.e.* captured at least 20 fish of each sex at a reference and an exposure site).

Table 1. Catch success for each Atlantic region mill in EEM Cycle One. "Method" is the capture method used, where 1=single-mesh-size gill nets, 2=multi-mesh-size gill nets, 3=electroshocking, 4=seining, 5=traps or trap nets, and 8=trawling.

Mill	Species	Method	Reference		Near-field		Far-field	
			Male	Female	Male	Female	Male	Female
Freshwater mills:								
Repap Groundwood NB	tomcod	5	21	19	8	20	10[1]	19[1]
Repap Kraft NB	tomcod	5	22	19	20	28	20	25
	white sucker		12	13	0	3	0[1]	0[1]
Fraser Edmunston NB	yellow perch	1	18	17	73	178	10	59
	yellow perch	1	20[1]	35[1]				
	white sucker	1	17	41	178	252	59	50
	white sucker	1	35[1]	63[1]				
Abitibi Grand Falls NF	threespine stickleback		21	20	15	25		
St-Anne Nackawic NB	white sucker	1	25	52	25	25		
	yellow perch	1	12	21	12	24		
Marine mills:								
Bowater Mersey NS	longhorn sculpin	gill nets	25	25	25	30		
	winter flounder	gill nets	2	1	25	23		
ABT Canexel	longhorn sculpin	2	20	25	12	25		
	winter flounder	2	24	25	24	25		
Irving Tissue St John NB	mummichog	5	20	20	7	7		
	tomcod	5	11	4	3	1	5	1
	tomcod	5	17[1]	7[1]	3[1]	2[1]		
	green crab	5	13	20	1	1	17[1]	18[1]
Lake Utopia NB	green crab	5	27	7	2	0	27[1]	32[1]
	tomcod	5	7	23	0	0		
Abitibi Stephenville NF	winter flounder	2	20	21	20	19		
	American lobster	SCUBA	20	20	20	20		
Corner Brook NF	winter flounder	2	26	30	36	47		
	cunner		20	20	19	21		
Boat Harbour Pictou NS	rock crab	5	20	20	20	20		
	winter flounder	8	9	34	4	36		
Stone Bathurst NB	tomcod	2	18	15	22	19		
	winter flounder	2	4	19	1	23		
Minas Basin Hantsport NS	tomcod	8	20	22	22	20		
	Atlantic silverside	8	60		60			
Stora Pt Hawkesbury NS	winter flounder	2	12	20	19	19		
	rock crab	2	20	11	20	20		
Avenor Dalhousie NB	winter flounder	2	12	26	12	21		

Irving paper St. John NB	rock crab	2	25	26	26	27		
	tomcod	5	7	17	0	5	2[1]	3[1]
	green crab	5	20	13	20	12	18[1]	17[1]

[1] These fish are from second and third reference sites

Problems encountered and results observed are summarized in Tables 2 and 3 respectively, and discussed in detail on a mill-by-mill basis in Part Two of this report.

Table 2. Problems encountered by marine/estuarine and freshwater mills in the Atlantic region.

Problem	Number of Marine/Estuarine Mills	Number of Freshwater Mills
Exposure not confirmed	8	2
Small gonads (immature or undeveloped)	6	1
Too few fish of one or both species	6	1
Plume small/deep/trapped/dissipated rapidly	5	0
Reporting unclear or incomplete	4	2
Only one sentinel species	4	1
Confounding factors	2	1
Reference site problems	2	1
Only juveniles captured	2	0
Migratory sentinel species	1	0

Table 3. Results observed for female fish populations in Cycle One Fish Surveys at Atlantic region mills (exposed relative to reference fish).

Parameter	Increase (%)	No difference (%)	Decrease (%)	Significant interaction
Age	2 (11)	14 (74)	2 (11)	0
Length at age	3 (16)	10 (53)	3 (16)	2 (11)
Weight at age	2 (11)	10 (53)	4 (21)	2 (11)
Gonad weight at body weight or length	2 (11)	11 (58)	0	4 (21)
Liver weight at body weight or length	6 (32)	10 (53)	1 (5)	1 (5)
Body weight at length	1 (5)	16 (84)	1 (5)	0
Fecundity	0	15 (79)	0	1 (5)

PART TWO: REVIEWS OF CYCLE ONE STUDIES FOR ATLANTIC REGION MILLS

Mill: ABT Canada Limited, Canexel Division, East River, NS

Problems:

1. Sampling may have occurred too early in the breeding season since gonads were not fully developed.
2. Both sentinel species used are migratory and no suitable tracer was available to determine long-term exposure of fish to the effluent (RFA analysis did not differentiate between exposed and reference fish).
3. Exposure zone is very small (diffuser is about 0.5 km offshore, 50 m deep). The plume is funnel-shaped, from submerged diffuser to surface.

Differences Observed:

Growth (weight-at-age, length-at-age) was increased in exposed longhorn sculpins. Liver weight (at adjusted body weight) was also increased in exposed sculpin of both sexes, and liver weight (at length) was increased in exposed female sculpin..

Reproductive parameters (gonad weight-at-adjusted body weight and gonad weight-at-length) were both increased in male sculpin from the exposure zone. The increased gonad weights of exposed sculpins had low *a posteriori* power values and were determined when gonads were not fully developed. Female longhorn sculpins showed significant interaction in gonad weight (at body length).
Growth of male winter flounder (weight and length at age) showed significant interaction, as did weight (at length) and gonad weight (at adjusted body weight).

EEM Cycle 2 Considerations:
Winter flounder should be replaced as a sentinel species.

Mill: Abitibi-Price, Grand Falls, NFLD

Problems:
1. Only one sentinel species (threespine stickleback) was available.
2. Only 15 males and 25 females from the exposure zone were used in statistical analyses.
3. Fish could not be properly aged because annuli were indiscernible on otoliths and other aging structures; length distributions (in literature) indicated that all fish were about one year old.
4. Gonads were very small (GSI ranged from 0.02-4.38 males; 0.01-21.68 females; most less than 1.0).

Differences Observed:
Growth: Reference females were longer than exposed females (but fish were not aged).
There were no other significant differences between the two populations.

EEM Cycle 2 Considerations:
Since only one sentinel species is used, larger samples could be processed. Seining or backpack shocking may be a more effective way of catching sticklebacks than either traps or boat shocking. A later sampling period may yield fish with larger gonads and should be considered.

Mill: Abitibi-Price, Stephenville Division, NFLD

Problems:
1. The exposure zone was not within the 0.1% effluent plume, because the plume is too small (~6 m from diffuser).
2. The reference site habitat differed from the study site.
3. No egg or fecundity measurements were available for either sentinel species because "no ovigerous females" were found. GSIs were low for female flounder (0.49-2.78), and for female lobster (0.19-0.43).
4. No condition indices were reported.
5. The report states that "testes were also larger at a given weight in the exposure zone" for lobsters, but the ANCOVA results indicate significant interaction in the ANCOVA of gonad weight at body weight. As with the female flounder and lobsters, the GSIs of male lobsters were low (0.07-0.38).

Differences Observed:
There were no significant differences for winter flounder.
Body weight (at carapace length) was heavier in exposed male lobsters than in reference males.
Exposed females were longer than references females, but lobsters could not be aged.
Hepatopancreas weight at body weight showed significant interaction for female lobsters.

Gonad weight at body weight showed significant interaction for male lobsters. Although gonad weight at body weight was not statistically different for female lobsters, GSIs of reference females were 0.46-4.30 % while GSIs of exposed female lobsters were 0.19- 2.70 %.

EEM Cycle 2 Considerations:
A more appropriate reference site should be found for this study. A different sentinel speceis is required; cunner, longhorn sculpin and small-bodied fish are possible alternatives. The timing of fish collection may need to be reconsidered in order to obtain sentinel species with more developed gonads.

Mill: **Avenor Maritimes Inc., Dalhousie, NB**

Problems:
1. Gonads were not fully developed in flounder and few egg cases were found on female crabs, indicating that the survey may have been too early in the year.
2. No flounder were processed from the current outfall location (some were caught but eaten by crabs before processed).
3. Historical deposition may confound results.

Differences Observed:
Growth (weight and length at age) of female winter flounder was increased at the future outfall site (exp2) relative to the reference site.
Energy storage: Male flounder were in better condition at the reference site relative to both current and future outfall sites (higher body weight at length). Reference male flounders also had heavier livers (at length). Plots of crab data indicate that crabs from all three sites are in similar condition.
Reproductive parameters differed among sites for male flounder, which had decreased gonad weights (at body length) at the future outfall area. Male crabs had increased gonad weights (at body weight) at both current and future exposure sites, relative to the reference site.

EEM Cycle 2 Considerations:
Cycle 2 data could follow the recovery of the Cycle 1 exposure zone. Cycle 1 data will provide a baseline for comparison of fish populations around the new outfall once it is in operation (after Dec. 1995). The timing of the survey should be later in the year if possible (early-mid fall) to allow flounder gonads to re-develop. If an increase in effort does not improve catch success, an alternative to the fish survey may be requried.

Mill: **Boat Harbour Wastewater Treatment Facility**

Problems:
1. Too few male flounder were caught, and flounder were difficult to catch in both zones. They may not be a suitable sentinel species in this study. No CPUE was reported but it appeared to be low.
2. Many female crabs were not gravid, so fecundity estimates were impossible. This is also true for some of the female flounder.
3. The fish and crabs captured in the exposure zone may not actually be exposed because the effluent is a thin layer floating on the surface. No effluent plume delineation or effluent concentrations were included in this report.
4. No indices (condition factor, LSI, GSI) were reported. Statistics were discussed but results were unclear.

5. Tracer analysis did not show any difference in RFAs, chlorophenols or salinity/TSS/colour between the two collection zones (at the bottom of the water column). No supporting measurements were reported (temperature, dissolved oxygen, etc.).

Differences Observed:
Few statistically significant differences were noted. Male crabs from the reference area had wider carapaces. Reference-area female flounder were older than exposure-zone female flounder. Some other differences were noted for female flounder between sites, but the reporting is unclear and no tabular summary of statitics is given.
EEM Cycle 2 Considerations:
The use of winter flounder as a sentinel species should be reconsidered because they are not resident in the study area, they are difficult to catch in reasonable numbers and *a priori* power analysis shows that they would be required in unreasonably high numbers in order to detect differences of even 50% (maximum of 36 males, 81 females required). Other fish to consider as sentinel species include sticklebacks and mummichogs or bivalves.

Mill: Bowater Mersey Paper Company Ltd., Liverpool, NS

Problems:
1. Too few winter flounder were available, so that only one sentinel species (longhorn sculpin) was used for statistical comparisons.
2. Gonads were not fully developed; sampling period may need to be later in the year.

Differences Observed:
Age of female sculpins differed, with exposed females significantly younger than reference females.
Gonad weight was significantly different between reference and exposed female sculpins. Exposed females had heavier gonads (at adjusted body weight) than reference females. This may not represent a real reproductive difference because gonads were not fullly developed at the time of sampling.
Energy storage: Exposed male and female sculpins had heavier livers (at adjusted body weight) than reference sculpins.

EEM Cycle 2 Considerations:
Winter flounder is not a suitable sentinel species for this site. Small-bodied species may be appropriate. The effluent plume at this location is relatively small.

Mill: Corner Brook Pulp and Paper Ltd., Corner Brook, NF

Problems:
1. Exposed fish may not be consistently exposed to the effluent.
2. On all maps in the report the outfall for the mill is labelled the "proposed outfall". The text implies that the mill was in operation when the study was conducted, and also states that the mill plans to install further treatment processes in the future. Was the study conducted on the basis of current mill operations at the time, or as a baseline study for monitoring changes after the planned improvements to wastewater treatment at the mill?

Differences Observed:
Growth differed for female winter flounder and female cunner (significant interaction for total length and body weight at age). Exposed female cunner had heavier livers (at body weight) than

reference female cunners. Exposed male cunner were longer and heavier at age than reference male cunner, and had heavier livers (at body weight).
Gonads were heavier (at body weight) in exposed male cunner than in reference male cunner.

EEM Cycle 2 Considerations:
Exposure to mill effluent of fish from the exposure zone should be confirmed through both plume delineation and tracers.

Mill: Fraser Inc., Edmunston, NB

Problems:

1. Many confounding factors are present at both reference and exposure sites, which make interpretation of results difficult (e.g. food processing plant, another paper mill, municipal discharges). For example, tracer studies (RFAs) appear to confirm exposure of fish from reference site 2 to mill effluent from Fraser Inc.'s Madawaska mill.
2. No plots of data were included and the raw data is disorganized. The discussion of the results is confusing and does not appear to deal with the statistical differences reported from ANCOVAs.
3. Range of ages of female white sucker is reported as 1.0-113.0 years. This is also in the raw data. If it is a mistake, it may have biased the statistical comparisons of growth and reproductive development.

Differences Observed:

Growth rates differed in yellow perch of both sexes and in female white suckers (fork length at age). Fish were generally older in the exposure zones. Also female white sucker populations differed in body weight (at length).

Gonad weight at body weight differed for male yellow perch (but reference fish were younger; mean age = 1.3 in reference, vs. 3.0 and 3.2 in the two exposure sites used). Fecundity (at age) differed between exposure sites and reference site 1 for female yellow perch. There was significant interaction for gonad weight at body weight of female white sucker and male yellow perch.

EEM Cycle 2 Considerations:

A second reference site on the Madawaska R. is needed for Cycle 2.

Mill: Irving Paper Inc., St John, NB

Problems:

1. Tracers (RFAs) do not confirm exposure/non-exposure of exposed and reference fish. The low levels of RFAs in exposed tomcod and the relatively high levels in tomcod from reference zone 2 (10 km away in Lorne Harbour) are not easily explained and may confound comparisons of populations to be made in Cycle 2.
2. Too few tomcod were captured from most sites. Catch data show that many more fish were caught than were retained but no explanation of this is given.
3. Confounding factors present in harbour.

Differences Observed:

ANCOVA results are given as raw computer output without labels as to species or sex, and no plots of data were included so conclusions are difficult to reach.

Growth: There is a significant difference in body weight (at age) between exposure site and reference site 2 for tomcod of one sex.

Energy storage: There is a significant difference in liver weight (at body weight) between exposure site and reference site 1 for tomcod of one sex.

Reproduction: There is a significant difference in gonad weight (at body weight) between exposure site and reference site 1 for tomcod of one sex.

EEM Cycle 2 Considerations:

Confounding factors such as other effluents and extreme tidal fluctuations make adult fish survey data from this site difficult to interpret. Caged mussels may be a better way to examine in-field effects at this site, and futher investigations into sublethal toxicity of the effluent should also also be considered instead of continuing the fish survey as conducted in Cycle 1.

Mill: **Irving Pulp and Paper (Kraft Mill) and Irving Tissue Company Complex, St. John, NB**

Problems:

1. Too few specimens were caught at nearly every site.
2. Tracers (RFAs) in fish tissue and in water failed to detect any difference between exposure and reference sites.
3. ANCOVAs were performed on data sets with as few as one fish (e.g. male tomcod from upstream exposure site, n=1; for females, n=4; ANCOVAs done for both these groups). This may affect the *a priori* calculations of required sample sizes.

Differences Observed:
Sample sizes were too small to give reliable ANCOVA results, and statistics were reported in an unclear manner. Ages of tomcod were the same at all sites but simple means indicate that male and female tomcod were longer and heavier at reference sites than at exposure sites for both upstream and downstream areas (except far-field downstream reference site for females). Egg volume of female tomcod was greater at the reference sites (n=3-17 at those sites). Mummichogs were not aged. Means indicate that male and female mummichogs were larger and heavier at reference sites and had heavier livers.
Green crabs were caught only at the downstream reference sites. The near-field reference sample had larger average carapace width and heavier bodies on average than the far-field reference sample. These size differences could be related to moulting stage; no mention in this report of controlling for moult stage.

EEM Cycle 2 Considerations:
A different sentinel species should be found. Three- and nine-spined sticklebacks were abundant at both upstream sites (upstream exposure and upstream reference) and may be a better choice for this reason. Also if they are available closer to the discharge, this may be a better site for the exposure zone (to be confirmed with RFA analysis).

Mill: **Lake Utopia Paper, St. George, NB**

Problems:

1. Too few of any species were caught to serve as sentinel species (in the exposure area).
2. The effluent plume was detected at different sites using measurement of true colour (TCU), but the TCU values were not related to specific dilutions of effluent. Furthermore, colour was the same at the three fish sampling stations (one exposure, two reference; TCU=3 or less). Exposure/non-exposure has not been confirmed.

Differences Observed:
Statistical comparisons were possible only for crabs and only between the two reference sites, since no fish were caught in sufficient numbers at the exposure site.

EEM Cycle 2 Considerations:
Locations of reference and exposure sites need to be re-evaluated. Perhaps fishing effort should be focused on other areas within Lake Utopia and in the impoundment. Knowing whether fish are present elsewhere in the lake but not near the outfall or in the impoundment, would be valuable information.
The marine environment, downstream of the mill, may be a more productive fish habitat than Lake Utopia and should be considered for the reference zone in Cycle 2 studies. A reconnaissance survey may be appropriate for the next cycle.

Mill: Minas Basin Pulp and Power /CFK, Hantsport, NS

Problems:

1. For one sentinel species (Atlantic silversides), only juveniles were captured. Adults are probably upstream in rivers during the sampling period of this study.
2. The effluent dissipates rapidly during high and intermediate tides, which are the only times fish would be exposed to it. The effluent plume is also bouyant at this time. Tracers confirmed exposure of fish only at the site nearest the boardmill outfall. The fate of effluent during low tide is unclear.

Differences Observed:
Reference tomcod (males and females) had lighter livers (at body weight) and lower condition factors than exposed tomcod.
Reproduction: Reference female tomcod showed significant interaction for gonad weight (at body weight), when outliers were removed.

EEM Cycle 2 Considerations:
Fish are hard to catch in sufficient numbers and are unlikely to be exposed because of a small plume and large tidal fluctuations. An alternative to the fish survey may be appropriate for future cycles.

Mill: Repap New Brunswick Kraft Mill, Miramichi, NB

Problems:

1. Too few white sucker were caught. Only one species (tomcod) was available for comparison.
2. No indices (condition factor, GSI, LSI) calculated.

Differences Observed:
Means show that all tomcod collected were about the same age (1.3-1.8 years) but that fish from the exposure site appear to be larger and have heavier gonads and livers than fish from either of the two reference areas. ANCOVA results show that there were significant differences in body weight (at length and age) but it is not clear which sites and sex are used in the comparison. There were no plots of data to facilitate comparison among sites.

EEM Cycle 2 Considerations:
A new sentinel species, such as mummichogs, should replace white sucker.

Mill: Repap New Brunswick Groundwood Mill, Miramichi, NB

Problems:

1. Only one species (tomcod) was abundant enough to use as sentinel species.
2. "Current" and "exisiting"outfalls are marked on the map but no indication is given of when the future outfall will come into use. Will it be within the Cycle 2 study period?

Differences Observed:
Very little variability is apparent in age and length of the fish at the three collection sites. The only statistical difference was in egg volume (at body weight) between the exposure site and the upstream reference site (reference site 1).

EEM Cycle 2 Considerations:
Mummichogs should be considered as an alternative sentinel species to white sucker in Cycle 2.

Mill: St Anne-Nackawic Pulp Co. Ltd., Nackawic, NB

Problems:

1. The habitats differed between the two zones.
2. Reference area fish may be exposed to effluent from upstream mills, and fish from the exposure zone may not be consistently exposed to effluent. Exposure-zone fish were not always collected within the 1 % plume because it was difficult to locate. Tracers (RFAs and dioxin/furan levels) were not substantially different in fish from the two zones.

Differences Observed:
Age: exposed male and female white suckers and male yellow perch were significantly older than reference fish. There were very few younger age class fish captured in the exposure zone.
Growth rates differed; exposed fish were smaller (length- and weight-at-age) than reference fish (both species and sexes). Energy storage also differed significantly, with reference fish having heavier bodies (at length) and livers (at adjusted body weight [ABW] or length) than exposed fish (for all groups except female perch, which had significant interaction or no differences).
Reproductive parameters of females showed significant interaction. Examination of plots indicate that reference females of both species have heavier gonads (at age and ABW). For males, gonad weight was greater in exposed males unless a reduced data set was used, which excluded fish with gonads weighing less than 10 grams.
Most differences noted were highly significant and with high *a posteriori* power (>0.8).

EEM Cycle 2 Considerations:
A more similar reference area should be sought.

Mill: Stone Container Ltd., Bathurst Division, Bathurst, NB

Problems:

1. All tomcod and all male winter flounder were immature fish (tomcod were all YOY).
2. Tracer (RFAs) in fish tissue showed no difference between sites.

Differences Observed:
Gonad weight of male tomcod differed significantly between reference and exposed sites. There was significant interaction for liver weight in male tomcod and for gonad weight in female tomcod. Liver weights of female winter flounder differed between exposure and reference sites, while most other parameters showed significant interaction, indicating that the two populations have different rates of growth and energy storage.

EEM Cycle 2 Considerations:
Different collection sites or methods should be investigated in an attempt to catch older tomcod and other species. Small-bodied fish may be appropriate sentinel species for future cycles.

Mill: Stora Forest Industries Ltd., Port Hawkesbury, NS

Problems:

1. Some possible confounding factors exist in the exposure zone, in the form of various industrial inputs and the use of the Strait of Canso for shipping. Furthermore the exposure zone was outside the extant 1% effluent plume at the time of the study, so that exposure was mainly through historical accumulation in the sediments, which does not accurately reflect the effects of current mill discharges. There was no successful tracer to determine exposure (RFAs were attempted).

2. No ovigerous rock crabs were captured, so no fecundity estimates were possible.
3. Too few male flounder and too few female crabs were collected.

Differences Observed:
Growth differed between sites for both species. Female rock crabs and female winter flounder were heavier (at shell width and age, respectively) in the reference zone. Male flounder were longer (at age) in the reference zone. Mean age also differed for male flounder.
Liver weight (at body weight) was greater in exposed female flounder than in reference females.
Male rock crabs showed significant interaction in dry hepatopancreas weight (at body weight).
Female rock crab had heavier hepatopancreas (at body weight) in the exposure zone.
Reproductive parameters differed significantly for male flounder (smaller gonads in exposed fish).
Male crabs showed significant interaction for gonad weight at body weight.

EEM Cycle 2 Considerations:
The data from Cycle 1 can be compared to data collected after implementation of secondary treatment and re-location of the diffuser to a site further offshore. Longhorn or shorthoen sculpin may be more appropriate sentinel species and different fishing gear may facilitate their capture.

Table 4 summarizes the data on each mill and the details of the Fish Survey as provided in the Cycle One report. Mills are listed in alphabetical order. "Changes Observed" are with respect to the reference site and are for statistically significant differences only, unless otherwise noted.

Table 4. Summary of Fish Survey designs and results for Atlantic region mills.

Mill & Location	**Type of Mill**	**Discharge environment**	**Receiver flow**	**Effluent flow**	**Approx. effluent conc.**	**Sentinel species**	**Collect'n method, dates**	**Numbers collected**	**Differences in exposed relative to reference fish**	**Problems**
ABT Canada Limited - Canexel Div., East River, NS	325 t/d hardboard products	2° trt then into mouth of Little East R., Mahone Bay (marine)	0.02-0.2 m/s; mainly tidal; dynamic effluent plume	2130 m^3/d	<=1% in 10-20 m from diffuser; up to 650m in	longhorn sculpin (*Myoxocephalus octodecemspinosus*)	longlines and gill nets (2.5-4.5" mesh); 6-15 Aug.,	20 M, 25 F ref; 12 M, 25 F exp.	F: +age, +body weight, +length, +liver weight, gonad weight SI M: +body weight, +length, +liver weight, +gonad weight	-gonads not fully developed, perhaps sampled too early (spawning december-february).
					worst case	winter flounder (*Pseudopleuronectes americanus*)	1995	24 M, 25 F ref; 24 M, 25 F exp.	F: no differences M: body weight, length, gonad weight all SI	
Abitibi-Price Grand Falls Div., NFLD	583 t/d of newsprint and specialty papers by TMP	partial 1° trt then Exploits R. via bank outfall	not reported	50000 m3/d; 0.597 m3/s	1% at diffuser, 0.33% shortly downstream	3-spined stickleback (*Gasterosteus aculeatus*)	trap nets (1 and 3" mesh), minnow traps (overnight), electroshocking (reference only); 6-11 Aug. 1995	21 M, 20 F ref; 15 M, 25 F exp.	all fish 1 yr. old; F: -length	gonads very small (most <1%); only 1 sentinel sp. and not aged
Abitibi-Price Inc., Stephenville, NFLD	500 t/d newsprint, by TMP	2° trt (ASB) then to St George's Bay, Gulf of St Lawrence	not reported	21000 m3/d	<1% in 6 m, <0.1% in 850 m	winter flounder	gill nets (2-5.5") and SCUBA divers	20 M,20 F ref; 21 M, 19 F exp.	no differences	- differences between ref and exp habitats
						lobster (*Homarus americanus*)	29 July - 4 Aug, 1995.	20 each sex, each zone	F: +width, hepatopancreas weight SI M: +condition, gonad weight SI	-report says exp. male lobsters had larger gonads; sig interaxn? -egg vol, fecundity, &

Appendix III

										indices not reported
Avenor Maritimes Inc., Dalhousie, NB	ground-wood and sulpho-nated chemi-mechanical	1^0 trt then dishcarge to Resti-gouche estuary (East Bay)	intertidal zone	56000 m^3/d	1% within 500m of outfall	winter flounder	gill nets (3-5" mesh)	14 M, 26 F, 51 tot ref; 10 tot exp1; 12 M, 21 F, 41 tot exp2.	F: +body weight, +length, +gonad weight (at age) M: -condition, -liver weight (at length), -gonad weight (at length)	-exp1=current outfall -exp2=future outfall -flounder gonads not developed, no fecundity measured
	pulp; 616.6 Adt/d of newsprint					rock crab (*Cancer irroratus*)	17-26 Aug., 1995	26 M, 26 F, 496 tot ref; 25 M, 25 F, 239 tot exp1; 25 M, 27 F, 836 exp2.	F: gonad weight SI M: +gonad weight	-few crabs found with egg cases
Boat Harbour Treatment Facility, Pictou Co., NS	bleached kraft sulphate mill	not reported	not reported	not reported	not reported	rock crab	crab traps, June 6, 1995.	20 M, 20 F each site	F: no differences M: +width	-too few berried crabs -too few male flounder
						winter flounder	otter trawl, Sept. 26, 1995.	9 M, 31 F ref., 4 M, 36 F exp.	F: -age; other significant differences but unclear; i.e. covariates used?	
Bowater Mersey Paper Co. Ltd., Liverpool, NS	TMP production of 590-885 Adt/d of softwood pulp for newsprint	untreated to Liverpool Harbour, estuary of Mersey R.; most dilution from tidal flushing, not	not reported	40 300 m^3/d in July 1995; 1995 mean = 32 200 m^3/d	1% at 500m seaward at low tide; 1% at 400m seaward at high	longhorn sculpin	long lines and gill nets (bottom sets in a bouyant plume); 25- July 1995.	22M, 16 F ref; 25 m, 30 F, 1 im exp.	F: -age, +liver weight, +gonad weight M: +liver weight	-gonads not fully developed, no conclusions on reproductive parameters -only 1 sentinel sp. available for stats
		Mersey R.			tide	winter flounder		2 M, 1 F, 1 im ref; 25 M, 23 F exp.	too few for stats	
Cornerbrook Pulp& Paper Ltd, NFLD	972 t/d newsprint by TMP; also recycling plant can produce	1^0 trt (dissolved air flotation clarifier) then to Humber Arm	not reported	80000 m^3/d into Humber Arm	not reported	winter flounder	gill nets, 0.5-4.0" mesh July 13-15, 1995	26 M, 30 F ref; 36 M, 47 F exp.	F: length and and body weight SI M: no differences	-report says tracer (RFA) analysis successful because DHA higher in exp fish; slightly higher, but why no other cmpds detectable?

	180 bone dry t/d of pulp									are exp fish really exposed?
						cunner (*Tautogolabrus adspersus*)		20 M, 20 F ref; 19 M, 21 F exp.	F: +liver weight, length and body weight SI M: +length, +body weight, +liver weight, +gonad weight	-why "proposed" outfall at mill? is this a future outfall? where is the current outfall?
Fraser Inc., Edmunston, NB	550 Adt/d sulphite pulp, 350 Adt/d ground-wood pulp,	2° trt then to St John R.	0.2 m³/s	not reported	not reported	yellow perch (*Perca flavescens*)	gill nets (2" mesh) and electro-shocking; 19-29 Sept. 1994	20 M, <30 F ref; <70 M, <70 F exp.	F: length, fecundity differed M: length differed, gonad weight SI	-mistake in data, one female white sucker 113 yrs old; effect on stats? -no plots shown
	120 Adt/d boxboard, 100 Adt/d de-inked news-papers					white sucker (*Catostomus commersoni*)		<35 M, <63 F ref; <178 M, <262 F exp.	F: length, condition differed, gonad weight and liver weight SI M: length SI	-significant inter-action ignored?
Irving Paper Inc., St John, NB	927 ADt/d	will have 2° trt, then discharge to Courtenay Bay, St. John Harbour	not reported	not reported	>1% in expo-sure zone, <1% in reference zones	tomcod (*Microgad-us tomcod*)	gill nets; 11 Oct. 1994 and 25-26 Aug. 1995	0 M, 5 F exp; 7 M, 17 F ref1; 2 M, 3 F ref2.	exp females all 2 yrs old (ref avgs 1.3-1.6); sig diff between exp and ref1 in liver and gonad wt (x body wt), don't know for which sex; same for body wt (at age), exp and ref2.	-many more tomcod adults caught, why not used? -most RFAs higher in fish from ref sites -no plots, outlier examination, etc.
						green crab (*Carcinus maenas*)		20 M, 12 F exp; 20 M, 13 F ref1; 18 M, 17 F ref2.	no differences	-p-values from ANCOVAs not reported -insufficient numbers of fish caught
Irving Pulp & Paper and Irving Tissue Company Mill Complex, St. John, NB	kraft pulp mill (810 t/d) and tissue mill (165 t/d)	Reversing Falls area of St. John R., approx 3 km upstream of river's mouth	not reported	10747 (tissue mill) and 64400 (pulpmill) m³/d	5% at near-field exp. sites, 1% at far-field sites	tomcod	trap nets; 23-26 Aug. and 11-18 Oct., 1995.	15 at f/w ref; 4 at upstr exp; 6 at downst exp; 24 at near ref; 5 at far ref	no reliable ANCOVA results but males appear longer, heavier at ref sites; females too except for far-field ref.	-why not use sticklebacks? -sample sizes too small; 10 out of 18 groups had <10 individuals

						green crab		2 downstr exp; 33 near ref; 35 far ref	ref sites only; larger carapace and liver at near-field ref than far-field ref	
						mummichog		40 at f/w ref; 14 at upstr exp.	not aged; appear larger, heavier at ref sites but similar liver wts	Mummichog not aged
Lake Utopia Paper, St. George NB	produces 450 ADt/d (of what?)	to Letang estuary (impound-ment)	not reported	not reported	9245 m³/d	tomcod	trap nets; 11-14 Oct., 1994.	0 ref 1; 7 M, 23 F ref2; 0 exp.		-too few exposed crab; comparisons between 2 ref sites only
						green crab		27 M, 7 F ref1; 27 M, 32 F ref2; 2 M exp.		
Minas Basin Pulp and Power/CKF, Hantsport, NS		Avon R. estuary, Bay of Fundy	tidal flow in Minas Basin	3693 m3/d and 3500 m³/d; 0.115 m³/s combined	<0.2% in 200 m, high tide	tomcod	otter trawl (1.27 cm mesh) July 6, 1994.	20 M, 22 F ref; 22 M, 20 F exp.	F: +liver weight, +condition, gonad weight SI (when outliers removed) M: +condition, +liver weight (when outliers removed)	-all silversides were YOY; too small for reprod. or internal organ measures, or for sexing
						Atlantic silversides		60 tot ref, 60 tot exp.	no significant differences; no reproductive measures	
Repap New Brunswick, Kraft Mill, Miramichi, NB	bleached kraft pulp and coated paper; 1307 ADt/d	Miramichi estuary	not reported	75000 m³/d	10% near-field; <1% far-field and reference sites	tomcod	gill nets; 20-21 Oct. 1994.	42 ref1; 47 ref2; 64 exp.	significant differences in weight (at lgth and age), can't tell which sex or sites used in comparison; means show exp fish are larger with heavier livers and gonads, but similar age.	-only tomcod in high enough numbers for comparison -SI for nearly all parameters in ref1 vs. ref2 and ref 2 vs. exp.
						white sucker		46 ref1; 0 ref2; 15 exp.		
Repap New Brunswick Inc.,	ground-wood mill;	Miramichi estuary	not reported	10100 m³/d	not reported	tomcod	gill nets; 29 Oct.-1	10M, 19 F ref1;	F: significant difference in egg volume (x body	

Groundwood Mill, Miramichi, NB	322 ADt/d						Nov., 1995.	20 M, 20 F ref2; 8M, 20 F exp.	wt) between ref1 and exposure sites	
St. Anne-Nackawic Pulp Co. Ltd., Nackawic, NB	bleached hardwood kraft pulp; 720 Adt/d (some ClO_2 subst'n)	2° trt then into Mactaquac L. (St John R.); lacustrine	486 m^3/s avg; 107 m^3/s low flow	7800 m3/d	1% in 400m avg river flow; 1% in 4km	white sucker	gill nets (3" mesh), October 1995	25 M, 25 F, 6 im, 156 total ref; 25 M, 25 F, 32 im, 186 total exp.	F: +age, -length, -body weight, -condition, -liver weight, gonad weight, egg size and fecundity SI M: +age, -length, -body weight, -condition, -liver weight, +gonad weight	-ref and exp areas dissimilar? -exp/non-exp not conclusively shown -gonads not heavier for exp fish when reduced data set used (<10g not included)
					in low flow	yellow perch		12 M, 22 F, 58 total ref; 12 M, 24 F, 1 im, 52 total exp.	F: -body weight, -length, gonad weight and egg size SI M: +age, , -length, -body weight, -condition, -liver weight (at length)	
Stone Container Canada Ltd., Bathurst, NB	BCTMP produces 250 ADt/y pulp, H_2O_2 for bleaching	near mouth of Nepisiguit R., up of Bathurst Harbour	not reported	645 t/d	up to 12% immed. below outfall; 0.5% at	tomcod	gill nets; 19-22 Sept. 1995.	18 M, 15 F ref; 22 M, 19 F exp. (all YOY)	F: gonad weight SI M: liver weight SI, gonad weight differs (outliers removed)	-too few male flounder
					2.2 km	winter flounder		4 M, 19 F ref; 1 M, 23 F exp.	F: liver weight differed M: too few for stats	
Stora Forest Industries Lt., Port Hawkesbury, NS	500 Adt/d each of bleached sulphite pulp and newsprint	1° trt then discharge to Strait of Canso, near shore	not reported	not reported	not reported	rock crab	gill nets (1-5.5" mesh); 20-24 June, 1995.	20 M, 11 F ref; 20 M, 20 F exp.	F: +condition, -liver weight M: +body weight, +length, gonad weight and liver weight SI	-higher temp in exp zone -should use ABW for rock crab, since body wt depends on hepatopancreas wt,
						winter flounder		12 M, 20 F ref; 20 M, 21 F exp.	F: -condition, +liver weight M: +age, +length, -gonad weight	which depends on moulting stage -confounding factors of exp zone, i.e. highly industrial, shipping

Appendix III

EEM CYCLE ONE FISH SURVEY REVIEW AND SUMMARY: QUEBEC REGION

PART ONE: INTRODUCTION AND REGIONAL SUMMARY

Forty-seven mills conducted Cycle One studies in the Quebec region. Forty-two discharge to freshwater (river or lake) environments and 5 discharge to marine/estuarine environments.

Determining catch success was considered a key element of Cycle One and catch success for the Quebec region is presented in Table 1. Ten out of 42 freshwater mills (11 if include unsexed smelt from Stone Port-Alfred mills) were successful for at least one species (*i.e.* captured at least 20 fish of each sex at a reference and an exposure site). Two out of 5 marine mills were successful for at least one species.

Table 5. Catch success for each Quebec region mill in EEM Cycle One. "Method" is the capture method used, where 1=single-mesh-size gill nets, 2=multi-mesh-size gill nets, 3=electroshocking, 4=seining, and 5=traps or trap nets.

Mill		**Method**	**Reference**		**Near-field**		**Far-field**	
			Male	**Female**	**Male**	**Female**	**Male**	**Female**
FRESHWATER MILLS:								
Maclaren Thurso	brown bullhead	3,2	20	20	20	20		
	yellow perch	3,2	21	20	20	20		
Domtar Lebel	white sucker	2,5	5	4	18	12		
	longnose sucker	2,5	11	14	7	9		
Domtar Beauharnois	white sucker	2	4	3	8	3		
	silver redhorse	2	3	7	1	4		
Tripap Trois-Rivieres		5,3	fewer than 10 of any species					
Stone Port-Alfred	smelt	2	40		40			
Stone Wayagamack	white sucker	2	5	8	7	9		
	shorthead redhorse	2	6	12	2	7		
Stone Pontiac	channel catfish	2	20	18	10	6		
	white sucker	2	4	13	7	4		
Abitibi Beaupres	longnose sucker	2	20	20	20	20		
Donohue St-Felicien	brown bullhead	2	20	20	20	20		
Alliance Donnacona	longnose sucker	1	20	20	20	20		
Cascades Breakeyville	longnose sucker	1	13	4	2	1		
BPCO Pont-Rouge	white sucker	1	20	20	3	10		
Cascades Jonquiere	longnose sucker	1	5	31	7	4		
Stone Laurentide	white sucker	2	6	8	3	8		
	longnose sucker	2	3	5	5	8		
Rolland St-Jerome	white sucker	3,1	25	28	22	30		
Cascades Cabano	white sucker	3,1	16	23	26	25		
	longnose sucker	3,1	0	0	14	28		
Kruger Trois-Rivieres	shorthead redhorse	1	1	3	2	0		
Cascades Kingsey Falls	white sucker	3,1	7	16	23	17		
	silver redhorse	3,1	18	22	20	20		
Cascades Joliette	white sucker	1,3	25	24	24	25		
FF Soucy R-du-Loup	white sucker	2,3	24	23	0	0		
Avenor Gatineau	white sucker	1	11	17	20	22		
Maclaren Masson	yellow perch	3	21	19	20	20		
	shorthead redhorse	3	2	2	21	20		
Cascades East Angus	white sucker	1	15	25	15	25		
	longnose sucker	1	0	0	20	22		
Desbiens	brown bullhead	5	0	0	1	2		

Kruger Bromptonville	white sucker	3	13	17	14	25		
Alliance Dolbeau	white sucker	gillnets	9	7	6	2		
	longnose sucker	gillnets	16	27	4	7		
Cartons La Tuque	white sucker	2	18	21	20	19		
	northern pike	2	2	5	6	6		
J. Ford Portneuf	white sucker	3	23	25	22	23		
	longnose sucker	3	22	22	22	21		
Scott Lennoxville	white sucker	3	20	24	23	22		
Scott Crabtree	white sucker	3	20	24	16	20		
Domtar Windsor	white sucker		40	40	34	37	37[1]	40[1]
Tembec Temiscaming	walleye	2	20	20	30	10		
Perkins La Chute		3,4	all immatures					
Abitibi Alma		3,4	all immatures					
Donohue Clermont	white sucker	2	20	20	7	3		
Donohue Amos	longnose sucker	1	21	20	20	20		
	brown bullhead	1	20	19	20	20		
Abitibi Kenogami	longnose sucker	1	7	4	51	48		
	white sucker	1	0	0	4	7		
Daishowa Quebec	no survey							
Mohawk St-Antonin	white sucker	1,5	21	17	21	6		
	yellow perch	1,5	2	21	0	6		
Malette St-Raymond	white sucker	2,5	13	24	27	3		
	longnose sucker	2,5	2	13	26	33		
EB Eddy Hull	white sucker	2	11	17	7	3		
Stone Belgo	white sucker	2		report not available				
MARINE MILLS:								
Abitibi Gaspesia	rock crab	5	20	20	20	20		
Stone Chaleurs	rock crab	5	20	20	20	20		
	winter flounder	5	10	30	12	12		
Quno Baie Comeau	rock crab	1,5	32	2	32	31		
Cartons Matane	rock crab	2,5	30	13	30	34		
Lupel CDM	white sucker		22	17	18	26		
Lupel CDM	shorthead redhorse		7	9	5	9		

[1] These fish are from a second reference site.

Problems encountered and results observed are summarized in Tables 2 and 3 respectively, and discussed in detail on a mill-by-mill basis in Part Two of this report.

Table 6. Problems encountered by mills in Quebec region.

Problem	Number of Mills
Exposure not confirmed	16
Too few fish of one or both species	25
Only one sentinel species	15
Small gonads (immature or undeveloped)	11
Confounding factors	11
Reporting unclear or incomplete	8
Plume small/deep/trapped/dissipated rapidly	2
Problems aging fish	2

Table 7. Results observed for female fish populations in Cycle One Fish Surveys at Quebec region mills (exposed relative to reference fish; 48 cases reported stats for most variables).

Parameter	Increase (%)	No difference (%)	Decrease (%)	Significant interaction
Age	5 (10)	35 (73)	8 (17)	0
Length at age	3 (6)	39 (81)	3 (6)	3 (6)
Weight at age	3 (6)	35 (73)	7 (15)	2 (4)

Gonad weight at body weight or length	6 (12)	30 (63)	8 (17)	2 (4)
Liver weight at body weight or length	8 (17)	29 (60)	6 (12)	4 (8)
Body weight at length	8 (17)	32 (67)	6 (12)	2 (4)
Fecundity	3 (6)	39 (81)	6 (12)	1 (2)

PART TWO: SUMMARIES AND REVIEWS OF CYCLE ONE STUDIES FOR QUEBEC REGION MILLS

Mill: Abitibi-Price, Alma PQ

Problems:
Only immature fish were caught.

Differences Observed: None

EEM Cycle 2 Considerations:
Attempts to catch adult fish in the main channel of the river should be made. Small-bodied fish species should also be considered as sentinel species.

Mill: Abitibi-Price, Beaupres PQ

Problems:

1. Tidal fluctuations cause reverse flow of plume during high tides and extremely low water in receiving area during low tides. The fish resident in the exposure area migrate daily to deeper water in the St. Lawrence River.
2. Only one sentinel species was used (LNS), although 60 walleye were caught in each zone; could they have been used as the second sentinel species?
3. The tracer was non-detectable in effluent, receiving water and LNS flesh.

Differences Observed:
Growth differed for female LNS (SI for length-at-age).

EEM Cycle 2 Considerations:
Small-bodied fish species should be considered as sentinel species.

Mill: Abitibi-Price, La Compagnie Gaspesia, Chandler, PQ

Problems:

1. Only one sentinel species was used (red crab). Over 600 were caught but only 20 males and 20 females from each zone were used.
2. Eggs were not counted because gonads were not mature enough.

Differences Observed:
Exposed female crabs had greater carapace width than reference females.

EEM Cycle 2 Considerations:
Crabs are abundant but cannot be aged, therefore are not an appropriate sentinel species. Bivalves and small-bodied fish could be considered instead.

Mill: Abitibi-Price Kenogami, Jonquieres, PQ

Problems:
1. Too few fish.
2. Difficulty aging fish.

Differences Observed:
Too few fish for statistics.
EEM Cycle 2 Considerations:
More than enough longnose sucker were captured in the exposed zone. More effort may be required to catch sufficient numbers in the reference zone. A new plume delineation will be required for the new outfall in Cycle 2.

Mill: **Alliance de Dolbeau**

Problems:
1. No statistics.
2. Too few fish; sampled in two different years.

Differences Observed:
None.

EEM Cycle 2 Considerations:
Incomplete report makes evaluation difficult. Yearly samples should not be pooled.

Mill: **Alliance, Donnacona PQ**

Problems:
1. Confounding factors include untreated sewage, dredging, tidal fluctuations and salinity.
2. Only one species (longnose sucker) was abundant enough in the exposure zone to be considered a sentinel species.
3. Male gonad weights were not reported.

Differences Observed:
No significant differences between reference and exposed fish. The graphs presented indicate significant interaction for liver weight (at body weight) and length (at age) for males and females.

EEM Cycle 2 Considerations:

Mill: **Avenor Inc., Gatineau PQ**

Problems:
1. Only one sentinel species was used.
2. Too few fish were caught in the reference zone.
3. Reference and exposure zone habitats differed.

Differences Observed:
Growth: Exposed male and female white suckers were lighter and shorter (at age).
Energy storage: Exposed males and females had lighter livers (at ABW) and lower LSI (at age).
Reproduction: Exposed males and females lighter gonad (at ABW) and lower GSI (at age).
Exposed females lower fecundity (at ABW); egg volume showed significant interaction.

EEM Cycle 2 Considerations:

The exposure zone was longer than the 1% field; delineation of 1% field and determination of exposure zone should be conducted again for Cycle 2.

Mill: BPCO, Pont-Rouge, PQ

Problems:

1. Too few fish were caught and only one sentinel species (white sucker) was used.
2. The river is a restored salmon river.

Differences Observed:
Statistical analyses were for females only (too few males). No statistical differences were found.

EEM Cycle 2 Considerations:
White sucker is a suitable sentinel species at this mill. The dams provide barriers to prevent exposure of upstream fish and those from further downstream. Greater fishing effort or a different fishing method may be required in the exposure zone, if this can be carried out without interfering with the salmon population. Otherwise, a non-lethal sampling of salmon juveniles could monitor the recovery of that population.

Mill: Cartons St-Laurent, La Tuque, PQ

Problems:

1. Too few northern pike were captured so only one sentinel species was used for statistical comparison (white sucker).
2. Log booming on the river causes habitat degradation (possible confounding factor).

Differences Observed:
The following conclusions apply to white suckers only.
Age: Exposed males were older.
Growth: Exposed males were shorter and lighter at age than reference males. Females showed significant interaction.
Energy storage: Exposed males and females had heavier livers at adjusted body weight than reference fish. Exposed males had higher LHI than reference males. Exposed females were lighter at body length than reference females.
Reproduction: Exposed males and females had lighter gonads at body weight and length than reference fish. Exposed males had lower GSI than reference males. Exposed females had lower fecundity at body weight and length and at age, and smaller eggs at age.

EEM Cycle 2 Considerations:

Mill: Cartons St-Laurent, Matane, PQ

Problems:

1. The effluent flow is small relative to the receiver flow. Confounding factors include other industries, storm sewer outfalls, fisheries, and tides (affect plume location).
2. No finfish were abundant enough in the exposure zone to use as a sentinel species. The red crab was the only sentinel species used.
3. Large numbers of red crab were caught in both zones, but only 13 females from the reference zone were used in statistical analyses.
4. Some female crabs had undeveloped gonads (GSI values less than one). Gonad measurements are missing for some reference zone females.

Differences Observed:
Age: Exposed female crabs were younger than reference females.

Growth: Exposed males and females had greater carapace width at age. Exposed females were lighter at age than reference females.
Energy storage: Exposed females were lighter at carapace width.
Reproduction: Female gonad weight at age, carapace width and adjusted body weight showed significant interaction. Exposed females were less fecund (at at age, carapace width and adjusted body weight) than reference females.

EEM Cycle 2 Considerations:
Crabs are abundant but cannot be aged, therefore are not an appropriate sentinel species. High dilution of the effluent makes exposure unlikely; caged bivalves could be considere at this site, but the effluent consists only of cooling waters after November 1995.

Mill: Cascades Cabano Inc., Cabano, PQ

Problems:
1. Too few longnose suckers (LNS) captured in the reference zone.
2. ANCOVAs were conducted using only total length as the covariable, despite weak correlations with other variables (e.g. $r^2 < 0.5$ for liver and gonad weight). Body weight and age should also have been used as covariates in ANCOVA analyses.

Differences Observed:
Statistics on white suckers only.
Age: Exposed males were older than reference males.
Energy storage: Exposed females had higher LSI than reference females.
Exposed fish were heavier and longer and had heavier livers and more eggs ($p<0.05$ in a t-test, but no ANCOVA of these parameters with age or body weight as covariate).

EEM Cycle 2 Considerations:
Many perch were caught by electrofishing in both zones; they should be considered for use as the second sentinel species in Cycle 2. Small-bodied fish should also be considered, since they are unlikely to use lake habitat which larger fish (*i.e.* the longnose suckers used in Cycle One) may be utilizing. The reference site location could be changed to account for the municipal effluent in the exposure zone.

Mill: Cascades East-Angus and Cascades Cartech Inc., East-Angus PQ

Problems:
1. Too few fish: no longnose sucker caught in exposure zone, too few male white sucker from both zones.
2. One third of the effluent flow consists of municipal sanitary treatment effluent.

Differences Observed:
Energy storage: Exposed males heavier liver (at body length). Exposed females body weight and liver weight (at length) showed significant interaction.
Reproduction: Exposed females heavier gonad (at body length).

EEM Cycle 2 Considerations:
A greater fishing effort may be required to obtain enough fish of at least one species. The municipal contribution to the effluent should be considered when interpreting future cycles.

Mill: Cascades Inc., Kingsey Falls, PQ

Problems:

1. ANCOVAs were conducted using only total length as the covariable. Body weight and age should also have been used as covariates in ANCOVA analyses.
2. Reference and exposure habitats differed.

Differences Observed:
Growth: Exposed male and female silver redhorse suckers (SRS) were older at length than in the reference zone.
Energy storage: Exposed male and female white suckers (WhS) were heavier at length. Male and female SRS showed significant interaction of body weight at length.
Exposed male WhS and SRS of both sexes had heavier livers at length; female WhS showed interaction of liver weight at length.

EEM Cycle 2 Considerations:
A more similar reference area should be sought.

Mill: Cascades Inc., Division Joliette, Joliette, PQ

Problems:

1. Only one sentinel species was used.
2. ANCOVAs were conducted using only total length as the covariable. Body weight and age should also have been used as covariates in ANCOVA analyses.
3. Mill effluent is about 1% of total effluent released from the combined mill and municipal outfall. The mill's process water is re-circulated, so that the effluent released is claimed to be non-toxic.

Differences Observed:
Growth: Exposed female white suckers (WhS) were older at length than reference females, and had higher condition and LSI than reference females. Exposed females also had heavier livers (a body length). Male white sucker showed significant interaction for liver weight (at body length). Exposed male white suckers had greater LSI and condition factor than reference males.
Reproduction: Exposed females had heavier gonads (at length). Reference males had higher GSI than exposed males.

EEM Cycle 2 Considerations:
Mill effluent is a small fraction of the total effluent released at this site. A wild fish survey may not be appropriate here.

Mill: Cascades Jonquiere, Jonquiere PQ

Problems:

1. Too few fish were caught. Only one sentinel species (longnose sucker) was used.
2. Male gonads were not measured.

Differences Observed:
No statistics because sample sizes were too small.

EEM Cycle 2 Considerations:
Greater fishing effort or different fishing methods are required for Cycle Two.

Mill: Daishowa, Quebec City, PQ

Problems:
No fish survey was conducted because collection was too dangerous in this area.

Differences Observed:
None.

EEM Cycle 2 Considerations:
The effluent makes up 0.01% of the total flow of the St. Lawrence River. Confounding factors include rapid dilution of the effluent, intense industrial activity, shipping, dredging and leisure activities as well as urban runoff and domestic inputs. Further studies should continue to focus on sublethal toxicity testing of effluent.

Mill: Desbiens

Problems:

1. Too few fish caught (3 mature brown bullhead were the only sentinels).
2. Presence of salmon limits sampling gear and time.

Differences Observed:
None.

EEM Cycle 2 Considerations:
The presence of Atlantic salmon in the Metabetchouane R. complicates fishing, which must avoid accidental catch of salmon. Different sentinel species (*e.g.* small-bodied species) and different fishing methods (*e.g.* beach seining) may avoid this problem.

Mill: Desencrages Cascades, Div. Rolland, Breakeyville, PQ

Problems:
Very few fish were caught and all were immature. The report states that the habitat in the study areas is better suited to juvenile fish than to adults (shallow, fast water, rocky substrate). A reasonable fishing effort was made (12 nets per zone for four days); however, only one type of fishing gear (gill nets) was used and it may not have been appropriate to capture what adults are there.

Differences Observed:
No statistics conducted.

EEM Cycle 2 Considerations:
22 species are known to inhabit the river. Different sampling gear and perhaps different locations should be attempted in order to catch sufficient adult fish (including small-bodied species). A juvenile fish survey may be appropriate.

Mill: Domtar a Lebel-sur-Quevillon, PQ

Problems:

1. Too few fish were caught.
2. There do not appear to be any fish available in the Quevillon R. (receiving water).

Differences Observed:
Age: Reference female longnose sucker (LNS) were younger than exposed female LNS.
Growth: Length and weight at age showed significant interaction for male white suckers (WhS)and plots indicated that reference fish grew more quickly.
Energy storage: Exposed male WhS had higher condition factor (body weight at length) and higher liver weight (at length) than reference male WhS.
Reproduction: Reference female LNS had higher gonad weight at body weight than exposed female LNS.

EEM Cycle 2 Considerations:
The survey should continue in the Quevillon R. if fish can be captured there. Other capture methods may be attempted, and other (non-benthivore) fish species may be considered as sentinel species.

Mill: Domtar Papiers Fine et Special a Beauharnois, PQ

Problems:
Too few fish were captured.

Differences Observed:
Statistics were conducted only on male white suckers and female silver redhorse suckers. No significant differences were found.

EEM Cycle 2 Considerations:
Other capture methods may be attempted, and other fish species (*e.g.* small-bodied species) may be considered as sentinel species in Cycle Two.

Mill: Domtar, Centre d'Affaires, Windsor PQ

Problems:
1. Only one sentinel species.
2. No measurements of male gonads.
3. Three upstream mills may create confounding discharges.

Differences Observed:
Energy storage: Liver weight (at body length) differs between exposed and reference females.

EEM Cycle 2 Considerations:
The reservoir area immediately upstream of the dam should be excluded from the reference zone in Cycle Two (fish further upstream if necessary).

Mill: Donohue Inc., Clermont, PQ

Problems:
1. Only one sentinel species and too few fish caught in exposure zone.
2. No suitable tracer found.
3. Presence of salmon limits sampling gear and timing.

Differences Observed:
No statistics.

EEM Cycle 2 Considerations:
Only gill nets were used (with variabe-size mesh). Other fishing methods might be attempted.

Mill: **Donohue Normick Inc., Amos, PQ**

Problems:
1. Problems aging fish lead to a negative growth slope in male exposed longnose sucker.
2. No tracer used.
3. Too few brown bullhead in reference zone (19 females).
4. Reference and exposure zones are close together with no physical barriers between.

Differences Observed:
Length at age differed for reference and exposed male lognose sucker.

EEM Cycle 2 Considerations:
The reference site may need to be located further upstream to ensure reference fish are not exposed. Small-bodied fish may be more appropriate species (smaller ranges).

Mill: **Donohue Inc., St-Felicien, PQ**

Problems:
1. Only one sentinel species was used (brown bullhead). Walleye were also abundant enough for use as sentinel species (caught 177 in reference, 58 in exposure zone) but were not used because they do not meet the criteria for sentinel species.
2. There is another mill upstream which discharges to the same river.
3. Lac Saint-Jean experiences yearly draw-down and other fluctuations caused by hydroelectric regulation of its tributaries.
4. Male gonad weights were not reported.
5. Reference and exposure zones were close together with no physical barrier between.

Differences Observed:
Energy storage: Females showed significant interaction for liver weight at body weight.

EEM Cycle 2 Considerations:
Use of a second sentinel species should be attempted. White suckers and perch are both present in the area and may be captured in sufficient numbers if different collection methods are used. A different reference site should be sought.

Mill: **E.B. Eddy, Hull, PQ**

Problems:
1. Too few fish.
2. Confounding factors: other discharges, rapid dilution of effluent, fast current, uneven and deep riverbed make fishing difficult.

Differences Observed:

No statistics.

EEM Cycle 2 Considerations:
Secondary treatment began after field work was completed; sublethal testing should continue to monitor improvements in effluent quality and may be considered for replacing AFS. Confounding factors include: other discharges, rapid dilution of effluent, fast current, and an uneven and deep riverbed.

Mill: **F.F. Soucy Inc., Riviere-du-Loup, PQ**

Problems:
1. Fish collection occurred in autumn 1991. Secondary treatment was implemented in July 1995 but no new fish collections were attempted at this time.
2. No fish were caught in the exposure zone.
3. The exposure zone is shallow and rocky and has dams, rapids and falls which make fishing difficult. The one depositional area where benthivorous fish such as white sucker may be found was fished with gill nets and electroshock but no fish were caught.
4. There are confounding factors such as industries, hydroelectric generating stations, farming and municipal inputs in the R. du Loup.

Differences Observed:
No statistics; fish collected from reference zone only.

EEM Cycle 2 Considerations:
The 1% plume extends as far as the mouth of R. du Loup at low river flows. Small-bodied fish species should be considered as sentinels in Cycle Two. Caged bivalves might be appropriate in the tidal area at the river's mouth.

Mill: **J. FORD Limitee, Portneuf, PQ**

Problems:
1. Five female white suckers used in reference zone data had undeveloped gonads - immature fish should be excluded from the analyses.

Differences Observed:
None.

EEM Cycle 2 Considerations:
The exposure zone is divided into two areas by a dam. Fishing should be from one area only.

Mill: **Industries James Maclaren, Masson PQ**

Problems:
1. Too few redhorse suckers caught.
2. Perch had undeveloped gonads.

Differences Observed:
The following apply to yellow perch only (too few redhorse sucker for stats).
Growth: Exposed of both sexes were longer and heavier at age.
Energy storage: Exposed males had heavier livers (at ABW). Both sexes were heavier (at length) in the exposed zone.

Reproduction: Exposed females had heavier gonads (at ABW). Exposed males had lighter gonads (at ABW).

EEM Cycle 2 Considerations:
Alternate fishing methods and a different reference site (further upstream in R-du-Lievre) may be required for this site.

Mill: Industries James Maclaren Inc., Thurso, PQ

Problems:

1. Tracers (RFA) were inconclusive in determining exposure/non-exposure of fish.
2. Most brown bullhead had undeveloped gonads (GSI<1). The six bullhead with GSI>1 were all from the reference area. It is unclear whether they were included in the statistical analyses (more fish are listed in the raw data than were included in the analyses).

Differences Observed:
Reference brown bullhead of both sexes were older than exposed bullhead.
Length (at age) was greater for exposed male yellow perch than for reference male yellow perch.
Condition factor (length at weight) was lower for exposed yellow perch of both sexes than for reference yellow perch. Liver weight at body weight was greater in exposed male perch than in reference male perch.
Gonad weight (at body weight) was greater in reference female brown bullhead than in exposed females.

EEM Cycle 2 Considerations:
Fish had GSI's smaller than 1%. Fishing may need to occur later in the year in order to sample fish with mature gonads.

Mill: Kruger Inc., Trois-Rivieres, PQ

Problems:

1. Too few fish were caught.
2. Reference and exposure zones are very close and there are no barriers in between, nor was there any data concerning tracers in fish (because too few were captured).
3. Three other mills and Reynolds aluminum are just downstream at Trois-Rivieres.
4. Fast current and deep water make fishing difficult.

Differences Observed:
No statistics.

EEM Cycle 2 Considerations:
The most abundant fish caught was the channel catfish (total of 8 caught). With a different fishing approach, these may be caught in sufficient numbers to use as a sentinel species. Fast currents, deep water, tides, and other inputs are problematic for study design.

Mill: Malette Quebec, St-Raymond de Portneuf, PQ

Problems:

1. Too few fish; no statistics on white sucker females or longnose sucker males.

2. No tracer was used.

Differences Observed:
Energy storage: Condition factor of male white suckers differed.
Reproduction: Fecundity (at body length) of female LNS showed significant interaction.

EEM Cycle 2 Considerations:
Fish had small GSIs (less than 1%); fishing may need to occur later in the year.

Mill: **Mohawk Pulp, St-Antonin, PQ**

Problems:
1. Too few fish of both sentinel species.
2. Statistics were conducted with fewer than 10 fish per sample but no power analyses were reported.
3. No tracer was used.

Differences Observed:
No statistics on male yellow perch (YP) because none captured in exposure zone.
Growth: Exposed male and female white sucker (WhS) were shorter and lighter at age. Exposed female YP were lighter but longer at age.
Energy storage: Exposed male WhS had lower weight at length than reference. Both sexes of WhS showed significant interaction for liver weight at body weight. Exposed female YP had lighter livers at body weight and showed significant interaction for body weight at length.
Reproduction: Exposed males WhS had lower gonad weight at body weight than reference. Female WhS showed signifiant interaction for gonad weight at body weight and for number of eggs per female. Exposed female YP had lighter gonads at body weight and more eggs per fish.

EEM Cycle 2 Considerations:

Mill: **Perkins Itee., La Chute PQ**

Problems:
1. No adult fish were caught, although lots of immature fish were.
2. Reference and exposure zones are close together with no physical barriers between.

Differences Observed:
No conclusions because no adult fish caught.

EEM Cycle 2 Considerations:
Small-bodied fish species should be considered for Cycle Two.

Mill: **QUNO, Baie Comeau, PQ**

Problems:
1. Only one sentinel species was used (rock crab), and only males were captured in sufficient numbers.
2. The effluent plume is highly variable (tides, winds) and no suitable tracer was found (RFA's are in low concentrations in the effluent).

3. Many crabs from both zones had GSI < 1.
4. The exposure zone in this study was new (diffuser installed Sept. 1995; study conducted Oct. 1995) so may not reflect effects of past effluent. This study may provide baseline data for continuing monitoring of crabs in the new exposure zone.

Differences Observed:
Energy storage: Exposed males had lighter livers at adjusted body weight, and lower LSI at age.
Reproduction: Male crabs showed significant interaction of GSI at age (no ANOVA of GSI alone).

EEM Cycle 2 Considerations:
Crabs are not a suitable sentinel species (cannot be aged). Use of caged bivalves may be considered at this site.

Mill: Rolland Inc., St-Jerome, PQ

Problems:
1. Only one sentinel species (but >20 per sex per site used).
2. Mill information included in proposal only, none in report.

Differences Observed:
Age: Reference male and female fish were older.
Energy storage: Condition factor was lower for reference males and females. Liver weight at body weight was heavier in exposed males and showed significant interaction in females.
Reproduction: Average egg weight was greater in reference females.

EEM Cycle 2 Considerations:

Mill: Scott Ltee, Crabtree, PQ

Problems:

Differences Observed:
The two sentinel species were white sucker (WhS) and silver redhorse sucker (SRS).
Age: Exposed male SRS were older than reference males.
Growth: Exposed male SRS were longer and heavier at age than reference males. Exposed male WhS were heavier at age than reference males. Female SRS and WHS showed significant interaction for growth parameters.
Energy storage: Exposed male SRS and WhS had greater liver weights at body weight and length than reference males. Exposed female SRS and WhS had greater liver weights at body length and greater LSI at age than reference females. Exposed male SRS showed significant interaction for LSI at age.
Reproduction: Exposed male WhS had greater gonad weight at age than reference males. Male and female SRS showed significant interaction for gonad weight at age. Exposed female WhS had lower GSI at age than reference females. Exposed female WhS and SRS had greater fecundity at age and adjusted body weight (and at length for WhS) than reference females.

EEM Cycle 2 Considerations:

Mill: Scott, Lennoxville, PQ

Problems:

1. No tracer was used because RFA concentrations are too low in the effluent. Since there is no physical barrier between reference and exposure zones and the two zones are 500m apart, exposure/non-exposure is not confirmed. (The reference and exposure zones were located according to TAP instructions.)
2. Only one sentinel species was captured (white sucker were captured in high numbers by electrofishing, while few other fish were caught).
3. Six reference female white sucker and one exposed female had GSI <1; most of these fish were under 4 years old and may have been immature.

Differences Observed:
Growth: Exposed males were longer and heavier at age (but r^2 was low for these regressions). Females showed significant interaction for these parameters.
Energy storage: Exposed females had heavier livers at adjusted body weight. Both sexes had greater LSI in the exposure zone.
Reproduction: Exposed males and females had heavier gonads at age and length, and showed significant interaction of gonad weight at adjusted body weight. Exposed females had greater GSI and greater fecundity at length and adjusted body weight.

EEM Cycle 2 Considerations:

Mill: Stone, Division Belgo, Shawinigan, PQ

Problems:
Final report not available, only the pre-study design and the appendices to the final report.

Differences Observed:
Sufficient numbers of fish were caught; appears (from appendix) that all variables are increased in the reference zone for both sexes and species, except for age and length, which are similar between zones for each sex and species.

EEM Cycle 2 Considerations:

Mill: Stone, Division Chaleurs, New Richmond, PQ

Problems:

1. Too few winter flounder used for statistics. Many more were caught than were measured but no explanation is given for this.
2. Gonads were not measured on male crabs.
3. Statistics reporting is incomplete; p values are given but the adjusted means and computer printouts of the analyses are not shown, nor is there any graphing of the regression results. Therefore it is impossible to tell which zone showed larger livers etc.

Differences Observed:
Rock crab showed significant differences in male hepatopancreas weight (at body weight) and female gonad weight (at body weight).
Winter flounder showed significant differences in male body weight and length at age, and female weight at age and condition factor.

EEM Cycle 2 Considerations:
Crabs are not a suitable sentinel species (cannot be aged).

Mill: Stone, Div. Laurentide

Problems:
1. Too few fish captured.
2. Other industrial and domestic inputs, and dredging.

Differences Observed:
Significant interaction for female white sucker length at age, body weight at age, condition and gonad weight at age and length (but n= 8 at both sites).
Male longnose sucker length and body weight at age significantly different (but n=3-5 fish per site).
EEM Cycle 2 Considerations:
Greater fishing effort or different fishing methods required for Cycle 2.

Mill: Stone, Division Pontiac, Litchfield PQ

Problems:
1. Too few of both sentinel species (channel catfish and white suckers).
2. The tracer (chloroguaiacols) were below detection limits in effluent, receiving water and catfish bile/liver. Furthermore the 1% plume is very small. Exposure of fish is not certain.
3. Most female channel catfish had undeveloped gonads.

Differences Observed:
No statistically significant differences were apparent in channel catfish from the two zones.
Growth: Reference male white suckers were longer and heavier at age than exposed male white suckers.
Reproduction: When one outlier was removed, there was a significant difference in gonad weight (at fork length) of female white suckers (but n=4 and 13 fish).

EEM Cycle 2 Considerations:
Alternate methods of capture should be investigated.

Mill: Stone, Division Port Alfred, PQ

Problems:
1. Fish were not sexed; statistical analyses were conducted on pooled samples of 40 fish per zone.
2. No measurements of liver weight, gonad weight or fecundity were recorded. The report states that the fish were too small to obtain these measurements, but mean total length for the reference and exposure samples were 18.77 and 16.67 cm respectively. This also confounds the significant result from the ANOVA on HAI, since no internal organ examinations were conducted in determining HAI.
3. No tracer was used in fish tissue because no liver tissue was recovered from the fish. Levels of the tracer (dehydroabeitic acid) were higher in the reference zone than in the effluent or receiving water.
4. American smelt (*Osmerus mordax*), the only sentinel species, are migratory. They probably enter the St. Lawrence in fall and overwinter there; therefore smelt caught in this area in October may not have been in the area for very long.

Differences Observed:
Age: Reference fish were older.
Growth: Reference fish had higher weight and length at age.
Reference fish also had higher condition factor.

EEM Cycle 2 Considerations:
Different means of capturing fish should be considered in order to obtain sufficient numbers of a species other than smelt, for use as a sentinel species.

Mill: **Stone, Division Wayagamack, Trois-Rivieres, PQ**

Problems:

1. Too few fish were caught (n=2 to 12 fish per site).
2. The reference and exposure zones are very near each other and the tracer used (DHA) was undectable in the effluent, water and fish tissue.
3. The text of the report states that the significant differences indicate larger livers in the reference zone (for example) but this is not shown either graphically or by reporting the adjusted means or the computer printouts of the statistical analyses. These should be included as evidence of the statistical findings.
4. There are a number of confounding factors including other discharges and heavy shipping traffic. The rapid currents and high water flows of the St. Maurice and St. Lawrence rivers lead to rapid dilution of the effluent.

Differences Observed:
The following are the results of ANOVA and ANCOVA analyses conducted with very small sample sizes (n=2 - 12 fish).
Energy storage: Male and female white suckers (WhS) had heavier livers (at body weight) in the reference zone. Exposed female shorthead redhorse (ShRS) had higher condition factor and heavier livers when outliers were removed.
Reproduction: Male WhS had heavier gonads (at body weight) and females had heavier gonads (at body weight, when outliers removed) in the . Exposed male ShRS had heavier gonads (at body weight) and reference female ShRS had higher fecundity (at body weight). Liver and gonad weights of female ShRS differed significantly when outliers were removed.

EEM Cycle 2 Considerations:

Mill: **Tembec Temiscaming, PQ**

Problems:

1. One sentinel species.
2. Too few walleye.
3. Most fish immature; no reproductive stats.
4. Exposed fish were collected in the plume of the North-South outfall (cooling waters), rather than in the plume of the treated effluent outfall.

Differences Observed:
Growth: Male length and body weight (at age) showed significant interaction.

EEM Cycle 2 Considerations:
Different fishing techniques may be required for cycle 2 (*e.g.* not experimental gill nets) to collect enough mature fish. Location of the exposure zone should be re-evaluated.

Mill: Tripap Inc., Trois-Rivieres, PQ

Problems:
1. Fewer than ten mature individuals of any of the potential sentinel species were caught.
2. Many confounding discharges are present and the effluent plume is small.

Differences Observed:
No stats were done because there were too few adult fish were captured.

EEM Cycle 2 Considerations:
Other capture methods may be attempted, and other (non-benthivore) fish species may be considered as sentinel species.

Appendix III

Table 8 summarizes the data on each mill and the details of the Fish Survey as provided in the Cycle One report. Mills are listed in alphabetical order. "Differences in exposed relative to reference fish" are for statistically significant differences only, unless otherwise noted.

Table 8. Summary of Fish Survey designs and results for Quebec region mills.

Mill & Location	**Type of Mill**	**Discharge envt.**	**Receiver flow**	**Effluent flow**	**Approx. effluent conc.**	**Sentinel species**	**Collection method, dates**	**Numbers collected**	**Differences in exposed relative to reference fish**	**Problems**
Abitibi-Price, Alma PQ	710 t/d paper by grdwd, bisulphite; also pulp purhased	2^o trt (ASB) then to R. Petite Decharge	not reported	48 000 m^3/d	not reported	only immatures captured	bourolles, seine (4 mm mesh), shocking; 28-31 Aug. 1995	All immature		-all fish immature
Abitibi-Price, Beaupres PQ	500 t/d specialty papers, H2O2 bleached	2^o trt then to r. des Vases			1% through-out R. des Vases	longnose sucker	Gill nets (5-9cm), shocking in exposure	20 M, 20 F ref; 20 M, 20 F exp.	F: length SI	-tracer unsuccessful -one sentinel sp. -confounding tide action
Abitibi-Price Kenogami, Jonquieres PQ	TCMP	2^o trt (ASS) since summer 1995 (moved diffuser too)	2-35 m^3/s during sampling in Saguenay	not reported	<10% in 100 m, <1% in ca.1200 m	longnose sucker	gill net (7.5 cm mesh); 18-26 Sept. 1995	7 M, 4 F ref; 51 M, 48 F exp.	no stats	-difficulty aging fish -no tracer -too few fish
		then to Saguenay and Sable R				white sucker		4 M, 7 F, exp. only	no stats	
Alliance de Dolbeau	CTMP	untreated	442 m^3/s	not reported	not reported	white sucker	gill nets, trap nets; 14-31 Oct. '94 and 22-29 Nov '95	9 M, 7 F ref; 6 M, 2 F exp.	no stats	-too few fish -sampled in 2 different years
						longnose sucker		16 M, 27 F ref; 4 M, 7 F exp.	no stats	
Alliance, Donnacona PQ	CTMP; 500 t/d news-print and specialty; H_2O_2 and Na hydro-	2^o trt (ASS) then to mouth of R. Jacques-Cartier at St.	85 m^3/s	ca. 29 000 m^3/d	<1% at 700 m up and down, due tides	longnose sucker	gill nets (7.5 cm) in exp; "fixed fisher" of a comm. oper-ation in ref; 11-16 Oct	20 per sex, per zone	F, M: no sig differences (but slopes show sig interaction?)	-untreated sew-age, dredging, tides, salinity -male gonads not measure

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	sulphate bleaching	Lawrence R.					1995			-BMPO mill 15 km upstr.
Abitibi-Price, Compagnie Gaspesia, Chandler PQ	700 t/d newsprint by CTMP, kraft and recycled	2^o trt then discharge to Baie des Chaleurs (coastal)	not reported	42 503 m^3/d	<1% at 700 m from outfall at low tide	red crab	traps; 5-8 Sept. 1995	20 per sex, per zone	F: +width M: no differences	-only 1 sentinel sp.
Avenor Inc., Gatineau PQ	1700 t/d newsprint; TMP and recycling	2^o trt (ASS) since June 1995	not reported	71.75 m^3/d	<1% at 1-11km (low-high river flows)	white sucker	gill nets (3-4" mesh); 25 Oct-24 Nov 1995	11 M, 17 F ref; 20 M, 22 F exp.	M: -length, -body weight, -gonad weight, -liver weight, -GSI, -LSI F: -body weight, -length, -condition, -liver weight, -gonad weight, -GSI (at age), -LSI (at age), -fecundity, egg volume SI	-only 1 sentinel sp. -too few from ref zone
BMPO, Pont-Rouge PQ	TMP and recycling	2^o trt (ASS) then to J-Cartier R., 100 m down of dam	25 m^3/s	not reported	<1% in 50 m	white sucker	7.5 cm mesh gill nets; 18-23 Oct 1995	20 M, 20 F ref; 3 M, 10 F exp.	F: no differences M: no stats	-too few fish -tracer unsuccessful (but dams present) -male gonads not measured
Cartons St Laurent, La Tuque	915 t/d kraft; 55% bleached	R. St-Maurice	not reported	125,000 m^3/d	1% in 800 m	white sucker	gill nets (1-4.5" mesh); 7-29 Oct 1995 (12 nights fishing)	18 M, 21 F ref; 20 M, 19 F exp.	F: -condition, +liver weight, - gonad weight, -fecundity, -egg volume M: +age, -body weight, -length, +liver weight, +LSI, -gonad weight (at length), -GSI	
						northern pike		2 M, 5 F ref; 6 M, 6 F exp.	too few for stats	
Cartons St-Laurent, Matane, PQ	320 t/d recycled cardboard (OCC)	1^o trt then St. Lawrence R. (estuary)	2500 m^3/d; 50-100 m/s	3000 m^3/d	<0.5 % in exp. by rhodamine dye study	rock crab	gill nets (5-6"), crab traps and palangres; 22-28 Sept. 1995	30 M, 13 F ref; 30 M, 34 F exp.	F: -age, -condition, +width, -fecundity, gonad weight SI M: +width	-tides, currents, winds -caught 193 crabs in ref but <15 females used in stats

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Cascades, Cabano PQ	not reported	not reported	slow	9-10 000 m^3/d	>3.5% at 885 m below outfall	white sucker	shocking from boat and gill nets (7.6-10.2 cm mesh);	16 M, 23 F ref; 26 M, 25 F exp.	F: +LSI M: +age	-graphs missing -too few LNS -ANCOVAs should use
						longnose sucker	13-16 Aug 1995	0 ref; 14 M, 28 F exp.		body wt as covariable
Cascades East Angus , Cascades Cartech inc, East-Angus PQ	not reported	not reported	not reported	10 000 m^3/d	<5% at ca. 1500 m	white sucker	gill nets (7.6-10.2 cm) and electroshock	15 M, 25 F ref; 15 M, 25 F exp.	M: +condition, +liver weight (at length) F: +gonad weight (at length), +GSI, body weight and liver weight SI	-some mill info missing
						longnose sucker	17-21 Sept. 1995	0 ref; 20 M, 22 F exp.		
Cascades Jonquiere, Jonquiere PQ	TMP and recycling	2^o trt (SBR) (sept 1995) then to R. aux Sables	23.2 m^3/s	not reported	not reported	longnose sucker	7.5 cm mesh gill nets; 19-25 Sept	5 M, 31 F ref; 7 M, 4 F exp.	F, M: no stats	-too few fish caught -male gonads not measured
Cascades Inc., Kingsey Falls, PQ	not reported	R. Nicolet Sud-Ouest	7.7 m^3/s	5700 m^3/d	>18% at 300 m from outfall	white sucker	shocking from boat and gill nets (7.6-10.2 cm mesh)	7 M, 16 F ref; 23 M, 17 F exp.	F: +condition, liver weigth (at length) SI M: +condition, +liver weight (at length)	-used length as covariable inst. of body weight -dissimilar habitats
						silver redhorse sucker	6-11 Aug. 1995	18 M, 22 F ref; 20 M, 20 F exp.	F: condition SI, + age, +liver weight (at length) M: condition SI, +age, +liver weight (at length)	-no info on mill processes
Cascades Inc., Div. Joliette	not reported	R. L'Assomption	not reported	1027 m^3/d	not reported	white sucker	gill nets (7.6-10.2 cm), trap nets, shocking; 30 Oct-2 Nov 1995.	25 M, 24 F ref; 24 M, 25 F exp.	F: +condition, +LSI, +age, +body weight, +liver weight, +gonad weight M: +LSI, -GSI, liver weight (at length) SI	-only one sentinel species -used length as covariable -no mill info
Desencrage Cascades, Div. Rolland,	115 t/d de-inked pulp from recycled newspapers	2^o trt (ASS) then to R. Chaudiere	1146 m^3/s	ca. 900 m^3/d	<1% in ca. 300 m	longnose sucker	verveux: 3.7 cm mesh, 3.3 m long, 0.75 m diam	13 M, 4 F ref; 2 M, 1 F exp; all	no stats	-only juvenile fish captured

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Breakeyville PQ	and magazines						8-11 Aug 1994	immature		
Daishowa, Quebec PQ	440 000 t/yr paper and 45 000 t/yr carton; TMP and desencrage	2° trt (UNOX) since Spet. 1995 then to St Lawrence R.	0.5-2.2 m/s (depends on tides)	86 700 m^3/d	not reported	no fish survey because too dangerous to collect				-effluent=0.01% of flow of river -industry, urban, shipping, port, dredging & leisure activities; tides
Desbiens	not reported	mouth of Metabet-chouane R., L. St-Jean	not reported	not reported	<1% in 75 m	brown bullhead	trappe-de-fond; 4-16 Oct 1995	0 ref; 1 M, 2 F exp.	no stats	-too few fish (22 total) -timing, method of capture
Domtar Fine & Specialty Papers, Beau-harnois	49.5 t/d recycled pulp and paper	2° trt then to Lac Saint-Louis	0.01-0.15 m/s	6041.3 m^3/d	<1% ~300m from outfall	white sucker	gill nets (6.2-10.2 cm) and trap nets,	4 M, 3 F ref; 8 M, 3 F exp.	F: no stats M: no differences	too few fish
						silver redhorse sucker	28 Sept - 5 Oct 1995	3 M, 7 F ref; 1 M, 4 F exp.	F: no differences M: no stats	
Domtar Lebel-sur-Quevillon	740 t/d bleached kraft pulp	1° trt then Quevillon R.	not reported	0.12-0.22 m/s	80% at discharge, 50% at mouth of Quevillon	white sucker	verveux, trap nets, gill nets (6.2-10.2 cm mesh),	5 M, 4 F ref; 18 M, 12 F exp	F: no stats M: +condition, +liver weight (at length), length and body weight SI, gonad weigth (at length SI),	
						longnose sucker	16-22 Sept 1995	11 M, 14 F ref; 7M, 9 F exp.	F: +age, -gonad weight M: no stats	
Domtar Windsor PQ	not reported	2° trt	not reported	not reported	<2% at 250 m	white sucker	shocking from boat and exper. gill nets 7-14 Sept. 1994	40 ea. ref1; 37 M, 40 F ref2; 34 M, 37 F exp.	F: liver weight (at length) differs M: no differences	-ref2 further up than ref1 -no male gonad wt -3 mills upstream
Donohue Inc., Clermont	ca. 1000 t/d newsprint	2° trt (ASS) since Aug. 1995; then	not reported	36 000 m^3/d	<1% at 10 km	white sucker	exerimental gill nets; 15-19 Aug	20 M, 20 F ref; 7 M, 3 F	No stats	-too few fish in exp zone -only one

PQ	by TMP and recycled	to R. Malbaie					and 10-13 Oct. 1995	exp.		sentinel species -no tracer
Donohue Normick Inc., Amos PQ	500 t/d newsprint by TMP	2^{o} trt (ASS) then to R. Harricana	not reported	29 000 m^3/d	<1% in 300-500 m	longnose sucker	gill net (7.5 cm mesh); 2-7 Sept. 1995	21 M, 20 F ref; 20 M, 20 F exp.	F: no differences M: length differed	-no tracer -problems aging fish lead to negative growth slope in exp male LNS
						brown bullhead		20 M, 19 F ref; 20 M, 20 F exp.	None	-ref and exp zone may be too close -difficulty aging fish
Donohue Inc., Saint-Felicien, PQ	kraft; 1030 t/d paper; ClO_2 (1992)	2^{o} trt (ASB) then 14 km away to mouth of Mistassini R (L. St. Jean)	not reported	not reported	up to 14.8% in receiving water	brown bullhead	2-7.5 cm mesh gill nets; 29 Sept - 7 Oct 1995.	20 per sex, per zone	F: liver weight SI M: no differences (gonads not measured)	-Alliance forest products upstr. -drawdown by hydro company -only one sentinel sp.
E.B. Eddy, Hull PQ	fine papers and tissue	1^{o} trt; 2^{o} trt began summer 1995; to Ottawa R.	1300-1359 m^3/s	not reported	usually <1% in several m	white sucker	gill nets (1.5-4") over night; 1-24 Nov. 1994	11 M, 17 F ref; 7 M, 3 F exp.	no stats	-rapidly diluted -too few fish, only one sentinel sp. -fast flow, deep uneven bed make fishing diffucult
F.F. Soucy Inc., Riviere-du-Loup, PQ	575 t/d newsprint from TMP	2^{o} trt (ASS) since July 1995	not reported		<1% in 1.5km (high river flow)	white sucker	gill nets (1-3" mesh) and electroshock ; autumn 1991.	24 M, 23 F ref; 0 fish exp.	no stats	-no exp. fish -confounding factors -different habitat exp/ref -poor fish hab?
J. Ford Ltee, Portneuf PQ	53.9 t/d various papers from purchased /recycled pulp	1^{o} trt then to R. Portneuf via 2 separate diffusers	5.2 m^3/s	4000 and 200 m^3/d for No. 1 and 2 diffusers, respect-ively	<1% in 90 m by rhoda-mine dye study	white sucker	electroshock 28 Sept-4 Oct. 1995.	23 M, 25 F ref; 22 M, 23 F exp.	F: -age, +body weight, +length, +liver weight, +LSI, +gonad weight (at age), -gonad (at length), +GSI, -fecundity, condition SI M: -age, +body weight, +length, +condition, +liver weight, +LSI, +gonad weight, +GSI	
						longnose sucker		22 M, 22 F ref; 22 M, 21 F	F: no differences M: -age, Length ad body weight SI	-5 imm. female WhS used in ref

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								exp.		
Industries James Maclaren Masson PQ	600 t/d newsprint; bisulphite pulp	1^o trt then to R. du Lievre	not reported	not reported	<1% at 1900 m from outfall	yellow perch	electroshock 23-30 Aug 1995	21 M, 19 F ref; 20 M, 20 F exp.	F: +length, +body weight, +condition, +gonad weight M: +length, +body weight, +condition, +liver weight, -gonad weight	-too few redhorse suckers caught -perch gonads undeveloped
						shorthead redhorse sucker		2 M, 2 F ref; 21 M, 20 F exp.	Some differences but too few fish	
James Maclaren Industries, Thurso PQ	620 t/d bleached kraft; Cl bleaching	1^o then to Outaouais R.	not reported	not reported	>1% in 800 m down of outfall	brown bullhead *Ameiurus nebulosus*	shocking (boat) and 5-9 cm mesh gill nets (exp)	20 each sex, each site	F:-age, -gonad weight M: -age	-tracers (chloroguaiacols) inconclusive -only 6 bullhead had GSI>1, all
						yellow perch	25-27 Aug. 1994	21 M, 20 F ref; 20 M, 20 F exp.	F: -condition M: +length, -condition, +liver weight	from ref (were they used in analysis?) -a few perch with GSI>1 also
Kruger Inc., Bromptonville PQ	not reported	R. St-Francois	<0.4 m/s	ca. 4500 and 27 000 m^3/d	not reported	white sucker	electroshock, gill nets, verveux	13 M, 17F ref; 14 M, 25 F exp.	None	-too few fish -could have used forage sp.
Kruger Inc., Trois-Rivieres PQ	1600 Mt/d paper	2^o trt then to St Lawrence R.	<1.5 m/s at ebb tide	72 000 m^3/d	>1% at 25 m from outfall	shorthead redhorse	trap nets and gill nets (7.6-10.2 cm mesh) 11-16 Sept 1995	1 M, 3 F ref; 2 M, 0 F exp	no stats	-too few fish -catch method? 2-6 trap nets, 1 gill net per zone at 10-14 m dpth
Mohawk Pulp, St-Antonin, PQ	ground-wood	1^o trt installed immed before study; effluent to	3 m^3/d (low flow)	not reported	not reported	white sucker	gill nets (2.5-3") and trap nets; 2-8 Oct. 1995	21 M, 17 F ref; 21 M, 6 F exp.	F: -length, -body weight, -fecundity, gonad weight and liver weight SI M: : -length, -body weight,-condition, -gonad weight, liver weight SI	-too few females but stats done anyway -too few male perch
		R-du-Loup				yellow perch		2 M, 21 F ref; 0 M, 6 F exp.	F: -body weight, -length, -gonad weight, -liver weight, condition SI,	-no power analysis -no tracer

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									+fecundity M: too few for stats	
Malette Quebec, St-Raymond de Portneuf, PQ	APMP; H_2O_2 bleach	2^o trt (ASS) since 1991, then to St-Anne R.	not reported	17 000 m^3/d	<1% in ca. 300 m	white sucker	gill nets (6.2-7.6 cm and exp.), trap nets	13 M, 24 F ref; 27 M, 3 F exp.	F: no differences M: condition differed	-no tracer -too few fish no stats on WhS females or LNS males
						longnose sucker	21-31 Aug. 1995 (7 d)	2 M, 13 F ref; 26 M, 33 F exp.	fecund (x lgth) SI	
Perkins ltee, La Chute PQ	50 t/d tissue, 57 t/d card-board and paper	2^o trt then to R. du Nord	not reported	1100 m^3/d	not reported	lots of immatures, no adults	bourolles, seine (4 mm), shocking; 16-19 Oct 1995.	All immatures caught		-caught lots of fish, all immature -ref and exp close together
Quno, Baie Comeau, PQ	1300 t/d newsprint by TMP and recycling	2° trt (ASS) since Sept. 1995; discharge to B. Comeau (St Lawrence estuary)	not reported	93 518 m^3/d	<8% in 1 km	rock crab	gill nets (5.5") and crab traps; 5-17 Oct 1995 (7 d fishing)	32 M, 2 F ref; 32 M, 31 F exp.	F: no stats M: -liever weight, -LSI, GSI SI	-only one sentinel species and not enough females
Rolland Inc., St-Jerome PQ	405 t/d fine papers	not reported	0.17 m/s	9 563 m^3/d	>= 5% in exposure zone	white sucker	shocking from boat and gill nets (7.6-10.2 cm mesh); 13-16 Aug 1995	25 M, 28 F ref; 22 M, 30 F exp.	F: -age, +condition, egg weight differed, liver weight SI M: -age, +liver, +condition	-only one sentinel species -sparse info on mill, production, etc.
Scott Ltee, Crabtree PQ	175 t/d tissue and hygienic paper; recycled pulp	2^o trt (ASS) then to R. Ouareau	13.9 m^3/s (mean summer)	20 516 m^3/d	<1% at 1 km (up to 65% at diffuser)	white sucker	electroshock at night; 11-21 Sept. 1995	20 M, 24 F ref; 16 M, 20 F exp.	F: +condition, +LSI (at age), -GSI (at age), +fecundity M: +body weight, +condition, +liver weight (at length), +gonad weight (at age)	-no com-parison of liver wt at ABW -no ANCOVAs of female gonad wt (M-W tests only)
						silver redhorse sucker		24 M, 24 F ref; 23 M, 23 F exp.	M: +age, +body weight, +length, condition, liver weight, gonad weight, LSI all SI	-some tests Mann-Whitney instead of

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									F: +condition, +LSI, +gonads weight, +fecundity, length and body weight SI	ANCOVA
Scott Ltee, Lennoxville PQ	53 kg/d Cottonelle toilet paper	1° trt then to R. Massawippi	9 and 55 m^3/s in Mass. and St-Francois rivers	4331 m^3/d	<1% in 1 km; up to 17% in Massa-wipi R	white sucker	shocking at night; 22-27 Sept 1995	21 M, 23 F ref; 23 M, 22 F exp.	F: +gonad weight (at length), +liver weight, +GSI, +LSI, +fecund (at length), length and body wieght and gonad weight SI M: +body weight, + length, +gonad weight (at length), +LSI, gonad weight SI	- no tracer, zones close together; exp. not con-firmed
Stone, Div. Belgo, Shawinigan PQ	CTMP	1° trt then to R. Shawinigan (3 diffusers)	3-27 m^3/s; 33 cm/s	53 403 m^3/d	15% at 500 m	white sucker, longnose sucker	gill nets (5-10 cm) for 7 days	seem to have >20 mature fish, both sexes and species	box plots show age and length similar, all other variables appear increased in reference zone	-other effluents (mill upstream)
Stone, Division Chaleurs	kraft; 626 t/d un-bleached cardboard	2° trt (ASB) after Cycle 1 work completed	not reported	not reported	not reported	rock crab	crab traps 15-21 Sept 1995	20 per sex per zone	F: gonad weight differed M: hepato-pancreas weight differed	-gonads not measured on male crabs -too few winter flounder caught
						winter flounder	5.5-6.5 " mesh gill nets; dates as above	10 M, 30 F ref; 12 M, 12 F exp.	F: body weight and condition differed M: length and body weight differed	-incomplete stats reporting
Stone, Div. Laurentide,	ground-wood sulphite	2° trt (SBR) (July 1995) then to R. St-Maurice	415 m^3/s (faster below dam)	758 t/d	not reported	white sucker	gill nets (2.5-10 cm mesh); 30 Sept - 7 Oct 1995.	6 M, 8 F ref; 3 M, 8 F exp.	F: length differed, condition, gonad weight, body weight all SI M: no differences	-other ind. and domest. inputs, dredging
						longnose sucker		3 M, 5 F ref; 5 M, 8F exp	F: M: length and body weight SI	-too few fish
Stone, Pontiac Division	kraft; 595 t/d bleached photogra-phic paper	2° trt (ASB) (1995) then to Outaouais R.	650 m^3/s Aug. 1994	60 959 m^3/d	0.5% in exp zone	channel catfish (*Ictalurus punctatus*)	2.5-10 cm mesh gill nets 23-29 Sept 1995	20 M, 18 F ref; 10 M, 6 F exp.	F: no differences but avg age=11.5 yrs in ref, 8.6 yrs in exp. M: no differences	-chloroguaiacol tracer unsuccessful -too few fish -immature

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	Cl, ClO_2					white sucker		4 M, 13 F ref; 7 M, 4 F exp.	F: no differences M: -body weight, -length	female channel catfish
Stone, Div. Port Alfred	TMP, CTMP, groundwood; 1035 t/d	2^o trt (SBR) then discharge to coast	not reported	64 460 m^3/d	varies with tide	American smelt (*Osmerus mordax*)	1-5" mesh gill nets overnight; 2-27 Oct 1995	40 ref; 40 exp; not separated by sex	F+M: -age, -body weight, - length	-no liver, gonad or fecundity data; no internal organ data in HAI -fish not sexed -fish are migratory
Stone, Div. Wayagamack, Trois-Rivieres,	TMP and kraft; 628 t/d specialty papers	2^o trt (SBR) then discharge to St Lawrence River	not reported	61 595 m^3/d	not reported	white sucker	1-5 " mesh gillnets 1-7 Oct 1995.	5 M, 8 F ref; 7M, 9 F exp.	F: condition and liver weight SI M: liver weight SI	-small sample sizes -ref and exp zones too close?
PQ						shorthead redhorse sucker		6 M, 12 F ref; 2 M, 7 F exp.	F: condition, fecundity, gonad weigth, liver weigth differed (hen outliers removed) M: gonad weight differed	-incomplete stats reporting
Tembec Temiskaming PQ	CTMP, bleached, and cellulose	2^o trt (ASS)	not reported	58 000 m^3/d	<2% at 500 m	walleye	experimental gill nets; 24-31 Aug 1995	20 M, 20 F ref; 30 M, 10 F exp.	F: no differences M: length and body weight SI, no stats on male reproductive parameters	-exp fish in plume of one of 2 outfalls; appropriate one? -few fish mature
Tripap Inc., Trois-Rivieres	520 t/d	2^o trt then to St. Lawrence R.	0.19-0.45 m/s	37 421.8 m^3/d	collections were wihthin visible plume	brown bullhead, white and longnose sucker, channel catfish, shorthead and silver redhorse sucker	5-12 Sept 1995	<10 of any sp or sex per site	No stats; too few fish	many confounding inputs, fast current, small effluent plume

EEM CYCLE ONE FISH SURVEY REVIEW AND SUMMARY: ONTARIO REGION

PART ONE: INTRODUCTION AND REGIONAL SUMMARY

Twenty-three mills conducted Cycle One studies in the Ontario region. All discharge to freshwater (river or lake) environments.

Determining catch success was considered a key element of Cycle One and catch success for the Ontario region is presented in Table 9. Twelve mills (13 if include unsexed spottail shiners from Iroquois Falls) were successful for at least one species (*i.e.* captured at least 20 fish of each sex at a reference and an exposure site).

Table 9. Catch success for each Ontario region mill in EEM Cycle One. "Method" is the capture method used, where 1=fixed-mesh-size gill nets, 2=variable-mesh-size gill nets, 3=electroshocking, 4=seining, and 5=traps or trap nets.

Mill	**Species**	**Method**	*Reference*		*Near-field*		*Far-field*	
			Male	**Female**	**Male**	**Female**	**Male**	**Female**
Sonoco Trenton	silver redhorse	2,3	25	22	20	20		
	shorthead redhorse	2,3	22	23	22	24		
EB Eddy Espanola	white sucker	2	15	3	16	3		
	walleye	2	11	4	23	2		
Sturgeon Falls	rock bass	2,3	17	18	18	11		
	spottail shiner	2,3	50		100			
Iroquois Falls	goldeye	2	23	17	20	18		
	walleye	2	20	20	20	20		
4 mills St Catharines	shorthead redhorse	3	40	39	39	23	68	79
Avenor Dryden	white sucker	2	5	20	21	20		
	walleye	2	16	17	21	20		
Stone Kenora	white sucker	2	14	25	25	23		
	walleye	2	25	20	3	0		
Stone Ft Frances	white sucker	2	10	9	24	26		
	walleye	2	20	7	22	6		
Avenor Thunder Bay	white sucker	2	20	20	20	21		
	longnose sucker	2	3	4	19	13		
Thunder Bay Pkg	spottail shiner	5	20	20	20	20		
Spruce Falls Kap	white sucker	1,3	16	15	27	24		
	walleye	1,3	9	3	7	2		
Provincial Papers	spottail shiner	2	20	20	20	20		
	white sucker	2	8	32	20	20		
Terrace Bay	white sucker	2	25	24	25	20		
	longnose sucker	2	13	15	17	23		
Strathcona Napanee	smallmouth bass	3	21	23	23	23		
	rock bass	3	23	24	22	23		
James River Marathon	longnose sucker	2	19	21	20	19	20[1]	21[1]
St Marys paper	white sucker	2	25	25	26	23		
	yellow perch	2	15	26	3	27		
Domtar Cornwall	white sucker		6	11	15	10		
	yellow perch		15	26	21	10	9[1]	27[1]
Domtar Trenton	carp	3	23	23	30	17		
	silver redhorse	3	26	25	23	13		
Domtar Red Rock	white sucker	1	49	43	29	51		
	longnose sucker	1	26	43	15	20		
Abitibi Ft William	spottail shiner	3	20	20	20	20		
Huntsville	fathead minnow	1,5	all immature					
Beaver Thorold	carp	3	8	10	25	20		
	pumpkinseed	3	22	20	30	28		
Malette Smooth Rock	white sucker	2	16	26	20	16		
	walleye	2	1	3	18	7		

[1] These fish were from a second reference site, not from a far-field exposed site.

Problems encountered and results observed are summarized in Tables 10 and 11 respectively, and discussed in detail on a mill-by-mill basis in Part Two of this report.

Table 10. Problems encountered by mills in Ontario region.

Problem	Number of Mills
Exposure not confirmed	6
Small gonads (immature or undeveloped)	4
Too few fish of one or both species	18
Plume small/deep/trapped/dissipated rapidly	2
Reporting unclear or incomplete	1
Only one sentinel species	3
Confounding factors	6
Reference site problems	7

Table 11. Results observed for female fish populations in Cycle One Fish Surveys at Ontario region mills (exposed relative to reference fish; 34 cases reported stats).

Parameter	Increase (%)	No difference (%)	Decrease (%)	Significant interaction
Age	2 (6)	24 (71)	6 (18)	0
Length at age	9 (26)	14 (41)	10 (29)	0
Weight at age	8 (24)	17 (50)	9 (26)	0
Gonad weight at body weight or length	5 (15)	20 (60)	8 (24)	1 (3)
Liver weight at body weight or length	11 (32)	17 (50)	3 (9)	3 (9)
Body weight at length	4 (12)	27 (79)	1 (3)	2 (6)
Fecundity	1 (3)	30 (88)	3 (9)	0

PART TWO: SUMMARIES AND REVIEWS OF CYCLE ONE STUDIES FOR INDIVIDUAL ONTARIO REGION MILLS

Mill: Abitibi-Price, Ft. William Div., Thunder Bay, ON

Problems:

1. Too few fish - only one sentinel species (spottail shiner), and from only one of two proposed reference zones.
2. The fish were too young (1 year) to give reliable gonad measurements, and this may affect detection of reproductive changes. For example, the largest average gonad weight was 0.0194 g. Furthermore eggs were not sufficiently developed to allow fecundity estimates (eggs could not be differentiated).
3. The mill discharges into a waterlot - difficult to find reference site.
4. Effluent form and upstream mill is present in the uptake water.

Differences Observed:

Growth parameters differed between reference and exposure sites. Fish of both sexes were larger in the exposure zone (possibly due to nutrient enrichment caused by the effluent).

Reproductive parameters were not significantly different although average female gonad weight was approximately 33% heavier in the reference zone than in the exposure zone (and reference females were smaller and slightly younger, although the age difference was not statistically significant).

EEM Cycle 2 Considerations:

Adult fish survey should be conducted again with one sentinel species and with older fish than were used in Cycle 1. Since the Cloud Bay reference zone was used in several Cycle 1 studies, mills could share fish data to maintain appropriate sample sizes and decrease fishing pressure in Cloud Bay. The mill might consider a combined survey with the upstream mill whose effluent is present in their uptake water.

Mill: Abitibi-Price Inc., Iroquois Falls, ON

Problems:
1. No fecundity measurements were available for walleye.
2. High incidiental catch.
3. Different habitat in reference and exposure zones.

Differences Observed:
Growth differed between sites with exposed male goldeye and exposed female walleye longer (at age) and exposed male walleye and exposed female goldeye heavier (at length).
Liver weights were higher in exposed males of both species (at body weight) and showed significant interaction in female walleye.
Gonad weight at body weight showed significant interaction for female walleye.

EEM Cycle 2 Considerations:
Forage fish may be appropriate sentinel species.

Mill: Avenor Inc., Dryden, ON

Problems:
1. Too few fish were collected from the reference zone (no statistics on white sucker; n=5 for males).
2. Fish capture was difficult; reference fish were bought from commercial fishers.
3. Because capture was difficult in the river which receives the mill effluent, the exposure site was in a lake located 81 km downstream of the outfall, where effluent was still >1%. Any changes that are seen between reference and exposed fish probably underestimate the effects of the effluent on fish in the river.
4. Different sized gill nets were used at the reference (4-5" mesh) and exposure (3-4" mesh) sites.

Differences Observed:
Growth differed for male and female walleye; both were substantially smaller (weight- and length-at-age) in the exposure zone ($p<0.00001$, power =1.0). Exposed females of both species had heavier livers (>30% heavier than reference females).
Gonads of exposed females were heavier (at ABW) than those of reference females for both sentinel species, and exposed female white sucker also had more eggs (at ABW).

EEM Cycle 2 Considerations:
Some important differences were found between exposed and reference fish. The AFS should be continued in Cycle 2, using the same sentinel species. Attempts to catch fish in the river should be made, even if gill nets cannot be used (as was the case in Cycle 1 when MNR would not allow gill netting in the river). Different sampling gear and timing should be considered.

Mill: Avenor Inc., Thunder Bay, ON

Problems:
1. Too few longnose sucker were collected, especially from the reference zone (therefore no stats performed for longnose suckers).
2. Dissolved oxygen was lower, temperature was higher and confounding discharges were more numerous in the exposure zone.

3. In the exposure zone, nets were set in two riverine and one lacustrine area. The location of capture may affect the outcome of the analyses, since feeding conditions may differ between the three exposed capture sites. Whether fish used in the analyses are from the river or how or the more lacustrine environment is not reported.
4. Tissue dioxin and furan levels did not differ in fish from the reference and exposed zones.

Differences Observed:
Reference white suckers of both sexes were older than exposed fish and age distributions were significantly different. There were fewer immature fish at the reference site which may indicate an older age-to-maturation at this site. Growth and condition appear to be enhanced in the exposure-zone fish (heavier and longer at age and heavier at length; highly significant and high power for both sexes). Liver weight did not differ between sites. Gonads in male white suckers were smaller (at body weight) in the reference zone (p=0.045, power=0.522), but female gonads were heavier (at body weight) in the reference zone (p<0.00001, power=1.0). Egg size was greater in reference females, while fecundity showed significant interaction.

EEM Cycle 2 Considerations:
Another tracer should be sought because the dioxin and furan analyses did not show any difference between exposed and reference fish. The Cycle 2 report should clarify from which net site within the exposure zone the fish used in analyses are collected an a riverine exposure area is needed. An alternate second sentinel species (small-bodied fish?) should be found to replace the longnose sucker, which were not collected in sufficient numbers.

Mill: **Beaverwood Fibre Company, Thorold, ON**

Problems:
1. Too few carp were caught in the reference area.
2. Nearly all reference-zone pumkinseed were four years old and nearly all exposure-zone pumpkinseed were two years old. This difference in age distributions confounds analysis of weight- and length-at-age and other parameters.
3. The area is heavily used for angling.
4. The mill has a partial closed-loop system.
5. A suitable reference area was very difficult to find.

Differences Observed:
ANOVAs and ANCOVAs were conducted on carp data despite small sample sizes in the reference zone (n=8 reference males and n=10 reference females).
Age differed for the two pumpkinseed (PKS) populations; mean age was older in reference males and females than in exposure fish, and age distributions were significantly different (most reference fish were four years old and most exposed fish were two years old).
Growth differed in carp. Reference males and females were heavier at age than exposed carp, and reference females were also longer at age.
Liver weight (at body weight) was higher in exposed female carp than in reference female carp. Exposed male PKS had a higher mean condition factor (before outliers removed) than reference male PKS.
Gonad weight (at body weight) was greater in reference male carp than exposed male carp.

EEM Cycle 2 Considerations:
A different sampling method may capture more carp and may select for the appropriate size/age range of pumpkinseed (e.g. appropriate size of gillnet, set overnight on the bottom, rather than electrofishing for carp in water >2 m depth). If no suitable reference site can be located, an alternative to the fish survey may be required for this site.

Mill: **Domtar Fine Papers, Cornwall, ON**

Problems:
1. Too few white sucker from all three sites.
2. The three sites (2 reference, 1 exposure) differ in habitat features (substrate, water velocity, bathymetry).

3. Dioxins and furans were higher in exposed white sucker but there were no differences in perch from the different sites.
4. There are many different inputs to the area, including Cornwall Chemicals and ICI, whose effluents are combined with Domtar effluent. Very fast currents cause rapid dilution of the effluent and cause difficulty for fishing.

Differences Observed:
Total weight (at length) differed for male yellow perch. No other statistically significant differences were found. Gonad weights were not significantly different but GSI values indicate that reference female yellow perch have larger gonads (at body weight) than exposed female yellow perch.

EEM Cycle 2 Considerations:
If appropriate reference sites which are more similar to the exposure site in physical features could be found they should be used instead of the reference sites used in Cycle 1. A larger-scale survey may be appropriate for this site.

Mill: Domtar Packaging, Red Rock, ON

Problems:
1. Too few male longnose suckers were captured at the exposure site (15).
2. There were no apparent differences in levels of dioxins/furans in fish from the reference and exposed sites. This does not confirm non-exposure of the reference fish; however the analyses of fish data show that the populations sampled differ significantly in many parameters measured, which indicates that they are indeed from two different populations.

Differences Observed:
Growth differed between the reference and exposed sites but there was no clear trend. Reference zone female white suckers were longer (at age) but exposed female white suckers were heavier (at age). Condition factor was slightly higher in the exposed zone for all groups except female longnose sucker (but no statistical tests on condition factor). Livers were lighter (at length and adjusted body weight) in reference fish, except in female longnose suckers where significant interaction between liver weight and its covariates was observed.
Heavier gonads were seen in reference fish for male (at length and ABW) and female white sucker (at ABW only) and for female longnose sucker (at length and ABW, after outliers removed). For male longnose sucker, exposure fish had heavier gonads (at length).

EEM Cycle 2 Considerations:
The sentinel species were easily obtained and power analyses show that differences of 20% can be detected using reasonable numbers of fish (N=2-60 except for age of male longnose suckers, where N=107). The AFS should be repeated at this mill to clarify the nature of differences that were observed in Cycle 1 (trends are not clear, e.g. gonad results differ depending on the covariate and species used). The same sentinel species and sampling sites could be used for Cycle 2.

Mill: Domtar Packaging Inc., Trenton ON

Problems:
1. Too few fish from exposure zone.

Differences Observed:
Age differed for female carp and male silver redhorse sucker.
Growth: Exposed male and female carp were longer at age than reference carp. Male carp body weight (at age) showed significant interaction.
Energy storage: Exposed male and female silver redhorse were heavier at length than reference fish. Reference male carp had higher liver weight at length than exposed male carp. Female carp liver weight at length showed significant interaction. Exposed male silver redhorse had lighter livers (at ABW) than reference; reference and exposed female silver redhorse showed significant interaction of liver weight and length.

Reproduction: Reference male carp had higher gonad weight at ABW (once outlier removed). Male and female silver redhorse showed significant interaction of gonad weight (at length and ABW).

EEM Cycle 2 Considerations:
Increased effort should allow successful capture in the next cycle.

Mill: E.B. Eddy, Espanola, ON

Problems:
1. Fish were difficult to catch at this site; too few females of both species were collected (9 female white suckers and 6 female walleye were collected in total). No statistics were performed on this data for females. Data from previous studies (Servos 1991-93) included females and were used for statistics and discussed in the report.
2. The Ministry of Natural Resources does not want the AFS to continue at this site (with walleye as sentinel species).
3. Reference sites are difficult to evaluate for this study.

Differences Observed:
Growth of male walleye was significantly different; fish were younger and larger at the exposure site. Livers of exposed male walleye and male white suckers were also larger (at fork length) than livers of reference fish, when outliers were removed.
Reproductive parameters of males of neither species differed significantly between sites. Gonads of exposed female white suckers were greater than reference females in the Servos 1991-93 data.

EEM Cycle 2 Considerations:
Extra effort should be made to include females, since a 1991-93 study (DFO) showed significant differences in gonads weights of females but too few were collected in this study to compare to previous studies. Alternate sentinel species could be evaluated using different capture techniques and alternate reference sites should be evaluated. Continuing downstream collections could use historical data as a reference.

Mill: James River-Marathon, Marathon ON

Problems:
1. Catches lake trout of were too low for statistical analysis.
2. Dioxins and furans seem elevated in fish from the reference zone although they are about 30 km away from the exisitng outfall at Marathon. Are there other inputs acting to confound these data?

Differences Observed:
Female longnose sucker at the future discharge site were younger than reference females.
Growth and energy storage: Males and females at the existing exposure site and females at the future exposure site were shorter at age than their reference counterparts. Males from the future discharge were lighter (at length) than reference males and showed significant interaction for liver weight (at ABW).
Reproductive differences were apparent in males from the existing exposure site, which had lighter gonads (at ABW) than reference males.

EEM Cycle 2 Considerations:
Lake trout may not be a suitable sentinel species for this location. Extra effort should instead be put into catching the longnose sucker, which are abundant in all three zones. A small-bodied fish species may be appropriate as the second sentinel species. A new reference site should be sought to ensure reference fish are not exposed.

Mill: Kimberly-Clark Inc., Huntsville, ON

Problems:
1. The habitat in the Big East R. is not suitable for fish and very few are found there (fast water velocity, sand bottom, few invertebrates and little instream cover). Therefore suitable numbers of adult fish were not obtained.
2. The mill discharges little effluent and subsequently has a very small plume area (1% dilution at 1 m from diffuser), increasing the difficulty of catching enough fish in the exposure zone.

Differences Observed:
No statistical analyses were carried out on fish because too few adults of any species were obtained. The fish

community is sparse within the Big East R. but more fish are more numerous and diverse at confluences with the oxbow tributaries of the Big East.

EEM Cycle 2 Considerations:
A small-bodied species such as the fathead minnow should be selected as sentinel species and the next cycle should continue to use Blackbird Creek as the receiving environment.

Mill: Kimberly-Clark Forest Products, Terrace Bay, ON

Problems:
1. No supporting measurements in reference zone, and no explanation.
2. Too few longnose suckers from reference zone (13 males, 15 females).
3. Report summary does not match their results, *i.e.* "general health assessment indices for both species were also identical for fish collected in the exposure and reference areas". In fact only fish from the exposed area were noted as having black spot, deformed caudal fin and cysts.
4. Dioxin and furan concentrations were higher in reference area fish when calculated as "maximum TEQ", but lower when calculated as "average TEQ". These measurements are very important as tracers and their relative concentrations in the two groups of fish should be as clear as possible. Raw data should have been included.

Differences Observed:
Growth was slower in exposed males white suckers. Exposed males of both species had heavier bodies and livers (at length) then reference males. Exposed females of both species had heavier livers (at age) but exposed white suckers were heavier (at length) then reference fish while exposed longnose suckers were smaller (at age) then reference fish. Reproductive differences were found only between reference and exposed female longnose suckers, which showed significant interaction with respect to gonad weight at age.
Fish were found in Blackbird Creek and Lake C for the first time since 1967. Most of these areas are 90% KCFP effluent, and the presence of fish there may reflect improved treatment of effluent and new secondary treatment introduced in 1989. Invertebrates are also more abundant in Blackbird Creek than when they were first noticed to be returning to the Creek in 1991.

EEM Cycle 2 Considerations:
Dioxin and furan levels in fish should be monitored again. Growth and reproductive parameters for fish should also be investigated again, using appropriate sample sizes as indicated by power analyses, for at least longnose sucker (the species which showed significant interaction in female gonad weights).

Mill: MacMillan Bloedel Ltd., Sturgeon Falls, ON

Problems:
1. No spottail shiners had fully developed gonads; mean ages of spottails were 1.0-1.2 years. Fecundity could not be determined for either species because gonads were too small.
2. Too few fish were captured, especially female rock bass (n=11).
3. The reference and exposure zones differed in habitat features (reference substrate was mainly mud, exposure zone substrate was mainly sand). Exposed fish have access to Lake Nipissing.

Differences Observed:
Growth: Male rock bass were older in the reference zone. Male rock bass and female shiners were longer (at age) in the reference zone. Female rock bass were heavier (at length) in the exposure zone and male shiners were heavier (at length) in the reference zone.
Liver weight (at body weight) of female rock bass was heavier in reference zone.
Gonad weight at body weight showed significant interaction for female rock bass and shiner.

EEM Cycle 2 Considerations:

This study was intended to provide baseline data against which future fish survey data could be compared. The next cycle of fish survey should use the same species, but an attempt should be made to collect more fish (min. 25 per site). Also, the reference site may be required.

Mill: Mallette Kraft Pulp and Power, Smooth Rock Falls, ON

Problems:

1. Too few walleye were collected (25 in the exposed zone, 4 in the reference zone).
2. No description of the reference site was included.
3. More white sucker were collected than were used in the anayses but the criteria for dropping fush from the data were not mentioned. There were four very small female white suckers (body weight < 375 g; GSI<0.65) from the reference zone that were included in the analyses but no small females from the exposed zone were included (all exposed female suckers had body weight>645 g and GSI>4.8).
4. The chloroguaiacols used as tracers in white sucker tissue were non-detectable at nearly all sites and did not clearly show exposure/non-exposure of fish from the two areas. However, walleye tissue was analysed for dioxin/furan content and fish from the exposed area had higher TEQs than reference fish.

Differences Observed:
There are no statistical results for walleye; the following conclusions apply to white suckers only.
Growth differed for male suckers, which were shorter (at age) in the reference zone, and for female suckers, which were shorter and lighter (at age) in the reference zone. Both males and females had higher condition factors in the reference zone than in the exposure zone.
Liver weight (at body weight) was higher for reference male suckers than exposed male suckers, but lower for reference females compared to exposed females.
Gonad weight (at body weight) was lower in exposed female suckers than reference females. For male suckers, gonad weight at body weight showed significant interaction. **Fecundity** (at body weight) was greater in reference female white suckers than in exposed female white suckers.

EEM Cycle 2 Considerations:
Alternate sentinel species should be considered to replace walleye. Reasonable numbers of longnose sucker were collected and they should be considered as an alternate sentinel species. Fixed mesh-size gill nets should be used to collect a more uniform, less variable fish sample.

Mill: Provincial Papers Inc., Thunder Bay, ON

Problems:

1. Reference and exposure sites differed in substrate and vegetation present. Temperature and dissolved oxygen also differed between the exposure and reference zone (lower temp., higher DO in reference zone).
2. RFAs were used as tracers but were not detectable in either zone.
3. Impacts of other local industries and intense navigation in the area are potential confounding factors.
4. Too few male white sucker were captured in the reference zone (n=8).
5. Spottail shiners were all 1 year old, with very small gonads and livers.

Differences Observed:
Species diversity was 16 in the reference zone and 5 in the exposure zone.
No ANOVA was conducted on ages of white sucker, which differ substantially between sites (reference males mean age=11.8 yrs, females=14.4 yrs; exposure males mean age=6.8 yrs, females=8.4 yrs). This appears to be due mainly to a lack of young fish in the reference zone, where minimum ages were 7 and 6 years for males and females respectively.
Growth was increased in exposed spottail shiners, with males and females longer (x age) and heavier (x length) than reference spottails. This increased growth may be attributed to warmer temperatures caused by effluent in the exposure zone.

Length-at-age showed significant interaciton in female white suckers. Energy storage: Exposed male spottail shiners and reference female white suckers had increased liver weights.
Gonads were heavier in reference white sucker females than in exposed white sucker females.

EEM Cycle 2 Considerations:
Sample sizes may need to be increased slightly in order to detect changes of 20% or more in gonad and liver weights of white sucker (N=28-239 for these parameters).

Mill: **Quno, Kimberly-Clark, Domtar and Thorold, St Catharines, ON**

Problems:
1. Only one species (shorthead redhorse) was abundant enough for use as a sentinel species, but these fish were readily available (sample sizes used in analysis ranged from 23-79 fish).
2. There are a number of confounding influences in 12 Mile Creek (other industries and combined sewer outfalls).
3. Gonads were very small in these fish (GSI was 0.5-2.0%). This may be due to the early sampling date (3-6 Aug.).

Differences Observed:
Growth: Near-field males were longer than far-field or reference males. Far-field females were heavier (at length) than near-field or reference females (marginal significance).
Liver weight (at body weight) was greater in fish from the near- and far-field sites than the reference site.
Gonad weight (at body weight) was lower in near-field males and in near- and far-field field females than in reference fish.

EEM Cycle 2 Considerations:
The sampling period should be later if possible. Sampling is difficult in this river (12 Mile Creek) and the sampling period was selected to coincide with yearly draw-down by the hydro operation upstream. It may be best to utilize only the near-field exposure site and the impoundment created by the hydro dam as the reference site 1.
Differences in growth and reproduction were apparent among the two populations sampled. Shorthead redhorse are readily available in all three study areas and the fish survey should continue using the shorthead redhorse as the only sentinel species.

Mill: **St. Marys Paper, Sault Ste. Marie**

Problems:
1. High flow volume and velocity of receiving water (St. Marys R.) provide quick mixing and dilution of the effluent, making fish sampling within the 1% effluent plume impossible. The exposure zone was located approx. 5 km downstream of the diffuser, well outside the 1% plume
2. Confounding factors: There are many other industrial inputs to the river upstream of the mill. There are also several municipal and WPCP outfalls in the study area. Tracers were used to determine exposure to metals (from Algoma Steel) and the mill effluent, but it is still impossible to separate the effects of the various inputs on the reference and exposure populations of fish.
3. Differences in habitat and accessibility to the lake may affect some parameters of the reference and exposure populations of yellow perch. Too few male perch were collected for statistical analysis.

Conclusions:
Growth rates of male white suckers differed between the two zones (significant interaction), but no young fish (<6 years) were caught at the exposure site so this is difficult to interpret. It may be that young suckers (<6 yrs) are too small in the exposure zone to be trapped in the gill nets used (no significant differences in size-at-age of male suckers but exposed female suckers and perch were significantly smaller-at-age). The report suggests food-supply differences between sites as a possible cause of size differences in female yellow perch; since the size difference was significant in all three groups analyzed (male and female suckers and female perch), food supply is not likely to be the sole cause of growth impairment.

Gonad weights were significantly different in male white suckers (larger in the reference zone) and female yellow perch (smaller gonads but larger eggs and greater fecundity in reference zone).
Liver weights were slightly enhanced for male and female white suckers from the exposure zone.

EEM Cycle 2 Considerations:
AN alternative to the fish survey should be considered for this mill since the confounding factors appear to be unresolvable.

Mill: **Sonoco Ltd., Trenton, ON**

Problems:

Differences Observed:
Age: Female silver redhorse were older in the exposure zone, while male silver and shorthead redorse were older in the reference zone. No silver redhorse older than 7 years were captured in the exposure zone, while silver redhorse in the reference zone were up to 13 years old.
Growth differed significantly between the two zones. Male silver redhorse were heavier in the exposure zone (when an outlier was removed) while male and female shorthead redhorse were larger in the reference zone. No significant differences existed in female silver redhorse.
Livers were heavier in male shorthead and silver redhorse from the exposure zone.
Gonads were smaller in the exposure zone for male shorthead redhorse (high significance and power).

EEM Cycle 2 Considerations:
For most parameters, sample sizes required to detect differences of 20% are reasonable (<30 fish) and for most of the remaining parameters, differences of 30% can be detected with fewer than 40 fish. Several significant differences were found in this study, which should continue with the same species in Cycle 2.

Mill: **Spruce Falls Inc., Kapuskasing, ON**

Problems:
1. Too few fish of both species were caught. No statistics reported for walleye (n=2-9).
2. No condition indices were calculated.
3. Habitat in the exposure and reference zones were dissimilar.
4. Means and box plots indicate substantial differences in ages (mean ages of reference male and female fish = 16 and 15 years respectively; mean ages of exposed male and female fish = 27 and 23 years respectively) and age distributions of white sucker populations. No ANOVA was reported for age and no mention was made in the summary.

Differences Observed:
Growth differed for female white suckers, which were longer (at age) and heavier (at length) in the exposure zone. No differences were detected in males.
Liver weight was increased in males and females in the exposure zone.
Gonad weight showed significant interaction for both sexes.

EEM Cycle 2 Considerations:
The AFS should be repeated with extra effort made to obtain minimum sample sizes for at least one species. Power analyses for white sucker indicate that 4-89 fish (but generally fewer than 30) would be needed to detect a 20% difference between sites, so sample size may have to be increased from 20 per sex per site to 30. Changing the timing of the survey may increase capture success.

Mill: Stone-Consolidated Corporation, Kenora, ON

Problems:

1. The exposure site had to be located downstream in a lacustrine environment, which contrasted with the more riverine habitat of the reference location.
2. Too few walleye were captured from the exposure for statistical analyses.

Differences Observed:
Growth differed in female white suckers (significant interaction for weight-at-age).
Liver weight at body weight was increased in exposed males and females (high significance and power). Liver weight at body length was also increased in exposed males.
Gonad weight was increased in male white sucker from the exposure zone, after outliers were removed (marginal significance and low power).
The lack of walleye from the exposure zone may be important in itself, especially considering the measurements of the three (male) walleye that were caught in that zone, relative to the 25 male walleye captured in the reference zone. Although no statistics were done, it can be seen from the raw data that although the exposed walleye were similar in length to the reference walleye, the exposed walleye had lower body weight, gonad weight, liver weight, GSI, LSI and condition factor than the reference walleye.

EEM Cycle 2 Considerations:
Although the lack of walleye in the exposure zone may be important, it may be inappropriate to use this species in the Cycle 2 FS. Instead, presence of walleye should simply be noted in the total catch data for both sites in Cycle 2. A lacustrine reference site should be sought.

Mill: Stone Consolidated Corporation, Fort Frances, ON

Problems:

1. The reference and exposure zone habitats were not similar.
2. Too few females were collected for statistical analyses.

Differences Observed:
Significant interaction for **growth** parameters (weight- and length-at-age) in male walleye were the only statistical differences found.

EEM Cycle 2 Considerations:
A change in timing may allow increased capture success with female fish.

Mill: Thunder Bay Packaging, Thunder Bay, ON

Problems:

1. This study was conducted as a baseline for future studies because the mill had been closed since 1991 and was due to begin operations in March 1996 (after the Cycle 1 AFS was completed). No information was given on planned processes or future water treatment at the mill.
2. Only one sentinel species was used (spottail shiner).
3. All fish were 1 year old, so gonads and livers were very small and the difficulty of dissection and measurement of these organs may lead to inaccuracy.
4. The reference and exposure zones are said to be similar in that both zones "were not affected by riverine water", but the Cloud Bay reference sites were near the mouth of the Cloud River.

Differences Observed:
Growth was increased in the reference zone, where females were longer (at age) than exposed fish. No difference was detected in liver weights.
Energy storage: Condition was increased in the reference zone in both sexes.
Gonad weight showed significant interaction for both sexes.

For all these differences, $p<0.001$.

EEM Cycle 2 Considerations:
Significant differences are attributed to habitat differences , since the mill was not in operation at the time of the study, but the study was in Thunder Bay, where numerous industrial and municipal inputs may be affecting fish at the exposure site.
Spottail shiner appears to be the only species available within the predicted effluent plume of the exposure zone, so this species should be used again in Cycle 2, and the same parameters tested. Comparison can then be made between exposed fish before and after mill operations begin, and between exposed and reference fish after operations begin.

Mill: **Strathcona Paper, Napanee, ON**

Problems:
Gonads of females were not well-developed.

Differences Observed:
Age: Exposed male smallouth bass (SMB) and exposed female rock bass (RKB) were older than reference fish.
Growth: Exposed male SMB were shorter and lighter (at age) than reference fish. Exosed female SMB were also lighter at age.
Energy storage: Exposed male SMB were heavier at length than reference males, and had higher LSI.
Exposed female RKB had higher LSI than reference females, and weight-at-length showed significant interaction.
Reproductive: Exposed female SMB had lighter gonads (at age) than reference fish. Exposed male RKB had lighter gonads (at ABW and body length) but significat interaction for gonad weight-at-age.

EEM Cycle 2 Considerations:
Sampling later in the year may allow collection of females with more well-developed gonads.

Table 12 summarizes the data on each mill and the details of the Fish Survey as provided in the Cycle One report. Mills are listed in alphabetical order. "Differences in exposed fish relative to reference" are for statistically significant differences only, unless otherwise noted.

Table 12. Summary of Fish Survey designs and results for Ontario region mills.

Mill & Location	**Type of Mill**	**Discharge environment**	**Receiver flow**	**Effluent flow**	**Approx. effluent conc.**	**Sentinel species**	**COLLECTION method, dates**	**Numbers collected**	**Differences in exposed fish relative to reference**	**Problems**
Abitibi-Price, Ft William Div., Thunder Bay, ON	400 tonnes/d standard newsprint (stone groundwood and chemi-mechanical pulping)	2° trt then into mouth of Mission R. at Thunder Bay; protected bay, sand substr.	not reported	0.16 m3/s	not reported	spottail shiner *Notropis hudsonius*	electro-shocking in exp and ref1, gill nets in ref2, trap and seine nets in all zones; 7-25 Sept. 1995		exp fish longer (at age), heavier (at length); exp males heavier liver (at ABW)	1. exp site warmer (13-24°C), lower O_2 2. fish too young to show reproductive differences? gonads almost too tiny to measure
Abitibi-Price, Iroquois Falls On, on Abitibi R.	ground-wood suplhite pulping for newsprint, specialty colour and	1° trt then discharge into Abitibi river via mid-channel diffuser	0-0.3 m/s	61 431 m3/d	not reported	goldeye *Hiodon alosoides*	gill nets, 6.35-12.7 cm mesh. 19-24 Sept 1995.	89 tot, 23M, 17 F 3 IM in ref; 49 tot, 20 M, 18 F 1 IM in exp.	F: +condition M: +length, + liver	
	weight papers					walleye *Stizostedion vitruem*	as above	47 tot, 20 M, 20 F 3 IM in ref; 63 tot, 20 M, 20 F 2 IM in exp.	F: condition differed; length, gonad weight and liver weight SI M: +condition, +liver weight, gonad weight and length differed	
Avenor Inc., Dryden ON	white paper products (300000 Adt/y), softwood BKP (285000 ADt/y)	2° trt then discharge to Wabigoon R., immed. downstream of dam	"slow"; fine particle substrate; 47-50.9 m3/d(1994 mean = 34.8)	110 000 m3/d	7.5% at 630m downstr; 1% at 81 km downstream (at Clay L.)	white sucker *Catostomus commersoni*	gill nets (3-4"), trap nets, 15 walleye purchased from	5 M, 20 F, 64 tot in ref.; 21 M, 20 F, 3 im, 51 tot in exp.	F: -age, +liver weight, +gonad weight, +fecundity, -egg volume, body weight and length SI M: no stats	1. no ref sites directly comparable to Wabigoon R. exp site b/c couldn't collect there; instead bought fish from Eagel L. commercial fishers
						Walleye	comm.	16 M, 17 F,	F: -age, -length, -	

							fishery, 20-27 Oct., 1994	3 im, 36 tot in ref; 21 M, 20 F, 7 im 133 tot in exp.	body weight, +liver weight, +gonad weight M: -length, -body weight	
Avenor Inc., Thunder Bay ON, on Kaministiquia R.	BK pulp (1500 tonnes/d) and newsprint (1500 tonnes/d)	2° trt for BKME, only 1° for newsprint (until Nov 1995); diffuser into Kamini. R; also littoral and limnetic habitat in	49.1 m^3/s; Sept 1994 avg =42.6	162 000 m^3/d; 1994 avg = 161000 m3/d	3% at 6 km (mouth of river), 1% at 8 km in avg. flow; 13% at low flow (Nov-Apr); 1% plume extends into	white sucker	gill nets (3-4" mesh) for approx 24 hrs, 19-26 Sept, 1994.	20 M, 20 F, 2 im, 66 tot in ref; 20 M, 21 F, 16 im, 61 tot in exp.	F: -age, +body weight, +length, +condition, -gonad weight, -egg volume, fecundity SI M: -age, +body weight, +length, +condition, +gonad weight	1. comparison for only 1 sentinel sp. 2. numerous anthropogenic inputs and higher temp in exp zone 3. no mention of use of tracer to determine if fish are actually exposed 4. how many exp fish are
		L. Superior			L. Superior	longnose sucker		3 M, 4 F, 3 im, 10 tot in ref; 19 M, 13 F, 22 im, 55 tot in exp.	too few ref fish for comparison; 4 ref females much older then exp females. Ref and exp males similar in age and other parameters	from each exp net site? (1 riverine, 1 near mouth, 1 in lake)
Beaver Wood Fibre Co., Thorold, ON		2° trt started after EEM study; discharge to Beaver Dams Creek	not reported	1183 m^3/d in Aug. 1995	up to 20% in east side of central arm of Lake Gibson	carp	shocking in dark; gill and trap nets unsuccessful;	8 M, 10 F ref; 25 M, 20 F exp.	F: -length, -body weight, +liver weight M: -length, -body weight, -gonad weight	1. too few carp from reference area 2. pumpkinseed have different age distributions
						pumpkinseed	14-18 Aug, 1995	22 M, 20 F ref; 30 M, 28 F exp.	ref fish older; exp males higher condition factor until outliers removed	confounds analysis
Domtar Fine Papers, Cornwall ON	640 Adt/d, BK (Cl, ClO2)	1° trt, combined with other industrial waste water then to St Lawrence	10-50 m^3/d	130000 m^3/d	<1% within 15 m	white sucker	gill nets and minnow traps, Oct. 4-8 and 18-23, 1993.	4 at ref1, 23 at ref2, 38 at exp	F: weight, gonad weight, liver weight, fecundity (at length) all differed, condition and length SI M: condition and liver weight differed, length	1. ref sites combined for analysis 2. no interpretation on stats

									and gonad weight SI	
						yellow perch *Perca flavescens*		53 at ref1, 132 at ref2, 78 at exp.	F: condition, gonad weight, liver weight (at length) all SI M: condition differed	1. ref sites combined for stats 2. no interpretation
Domtar Packaging, Red Rock, ON	850 t/d un-bleached kraft liner	Nipigon Bay, just down of mouth of Nip. R.	not reported	83300 m^3/d	<1% beyond 20 km in any direction from mill	white sucker	4-4.5" mesh gill nets, Sept. 26-Oct. 2,	49 M, 43 F ref; 29 M, 51 F exp.	F: -gonad weight, +liver weight, -length, +body weight, -fecundity M: -gonad weight, +liver weight	
						longnose sucker	1994.	26 M, 43 F ref; 15 M, 20 F exp.	F: +length, -gonads (outlier removed), body weight and liver weight SI M: +body weight, +length, +gonad weight, +liver weight	
Domtar Packaging Inc., Trenton, ON	425 t/d corrugating medium from wood	Trent R., rock substr. with some sand	1992 mean = 113 m^3/s (11.4-305)	0.0477 m^3/s, or 4120 m^3/d	1% within 500m	carp	electro-shocking, 30 Aug.-2 Sept. 1994	30 M, 17 F exp; 23 M, 23 F ref1	F: age, +length, liver weight SI (at length) M: +length, gonad weight and liver weight differ, condition SI	1. too few fish
	and recycled paper					silver redhorse		23 M, 13 F exp; 26 M, 25 F ref2	F: +condition, -fecundity, gonad weight and liver weight SI M: -age, +condition, -liver weight, gonad weight SI	
E.B. Eddy, Espanola ON, on Spanish R.	BKPM, specialty and fine papers	2° trt. (ASB) then discharge through submerged diffuser into Spanish R.	130 m^3/d (annual mean); 69 m^3/d m^3/d typical monthly low flow	1.36 m^3/s; 118000 m^3/d	not reported	white sucker	gill nets, 3 and 4" mesh, overnight; 9-16 Oct. 1994	15 M, 3 F 7 IM ref; 16 M 3 F 2 IM exp.	F: no stats M: +age, +Liver weight (outliers removed)	1. too few females of either sentinel species
						walleye	as above, deeper water	11 M 4 F 2 IM ref, 23 M 2 F 35 IM exp.	F: no stats M: -liver weight (at length; outliers removed), -age, + body weight, + length, +GSI (at age)	

James River-Marathon Ltd., Marathon, ON	BK pulp, 485 Adt/d; 100% ClO_2 bleaching	2° trt (ASB) intro'd after study, discharge via diffuser to L. Superior	lacustrine with rubble, sand and cobble substrate; mud further lakeward	44330 m^3/d during study; 43015 m^3/d 1994 avg.	existing: 2% initial, 1% for $1km^2$. future: 2% initial, 1% for 300 m^2	longnose sucker	3-4" gill nets overnight, 7-14 Sept. 1994.	19 M, 21 F, 4 im, 80 tot in ref; 20 M, 21 F, 7 im, 75 tot future exp;	F: -age, -length, -body weight, -gonad weight (at age) M: -condition, liver weight SI (ref vs. future exp)	1. only one sp abundant enough for sentinel. 2. tracer results are confusing
								20 M, 19 F, 3 im, 68 tot at existing exp.	F; -length, -body weight, -gonad weight (at age) M: -length, -body weight, +liver weight (outlier removed (ref vs. current exposure)	
Kimberly-Clark, Huntsville, ON	tissue mill using purchased pulp	Big East R.	6.29 m^3/s low flow	0.00505 m^3/d	1% at 1m, 0.19% within 4.5 m	fathead minnow and common shiner	gill nets (2-2.5 cm mesh) and minnow traps; 2-5 and 16-17 May 1994		too few adults of any species for stats; unsuitable fish habitat; caught more fish at mouths of oxbow tribs (but well of outside effluent plume)	1. CPUE= 0.3 for Big East R., 4.3 for oxbow tribs at confluence with Big East R.
Kimberly-Clark; Terrace Bay ON	1279.7 Adt/d bleached kraft (Cl bleaching) sulphate	1^o and 2^o trt then into Blackbird Crk	0.7 m^3/s	1.25 m^3/s	not reported	white sucker	gill nets, 3-4" mesh, left 20-24 hrs (overnight)	25M, 24 F, 1 im, 145 tot ref; 25M, 20F, 4im, 58 tot exp	F: +condition, +liver weight (at length) M: growth SI, +condition, +liver weight	1. no supporting msmts in ref zone
	pulp					longnose sucker	22-28 Sept. 1995	13 M, 15 F, 1 im, 30 tot ref; 17 M, 23 F, 3 im, 87 tot exp.	F: -body weight, -length, +liver weight (at age), gonad weight SI (at age) M: +age, +condition, +liver weight (at length)	
MacMillan Bloedel, Sturgeon Falls, ON	corrugating medium from OCC re-pulping	tailrace of power gen. plant then Sturgeon R.; but study was in receiving	not reported	3000 m^3/d	not reported	rock bass *Ambloplites rupestris*	gill nets (2-4.5") near shore, boat shocking near shore and funnel	17 M 18 F 3 IM? Ref; 18 M 11 F 6 IM? exp.	F: +condition, -liver weight, gonad weight SI M: -age, -length	1. ref substrate predom. mud, exp predom. sand. 2. also most exp fish were collected outside the
		area of local WPCP, 1.5 km				spottail shiner *Notropis*	traps; 18-28 Sept 1995.	total of 50 at ref.	F: -length, body weight and gonad weight SI M: -condition	WPCP plume and in effluent at < 100:1 dilution so

		downstr.of mill				*hudsonius*		>100 at exp.		diffs not likely due to mill effluent.
Malette Kraft Pulp and Power Co., Smooth Rock Falls ON		2^0 trt (ASB) then discharge to Mattagami R.	not reported	0.8 m^3/d	vert mixed at 200m; 1.29 % at 14.4 km	white sucker	gill nets (6.35-12.7 cm mesh) overnight, 21-25 (?) Oct. 1995	16 M, 26 F ref; 20 M, 16 F exp.	M: -condition, -liver weight, +length, gonad weight SI F: +length, +body weight, -condition, -fecundity, gonad weight SI	1. too few walleye 2. small female suckers included in ref analyses only? 3. no description or detailed location of reference site
						walleye	as above	1 M, 3 F ref; 18 M 7 F exp.	no stats	("above mill")
Provincial Papers Inc., Thunder Bay ON	460 tonnes/d of fine coated paper	1^o trt then Lakehead Harbour	not reported	33 428 m^3/d 0.347 m3/s	>1% in exp zone	spottail shiner	gill nets (2-4.5" mesh) and trap nets 64 mm mesh); also seine net in ref.	79 ref, 455 exp; in ref and exp, 20 M and 20 F used	F: +length, +condition, gonad weight SI M: +length, +condition, +liver weight	Habitat differences, tracers not found in exp fish flesh,
						white sucker	10-24 Sept. 1995.	72 ref, 74 exp; in ref 8 males, 32 females used; exp 20 M and 20 F used	F: -gonad weight, -liver weight, length SI M: no differences	
Quno, Kimberly-Clark, Domtar and Thorold;	St. Catharines and Thorold, ON	Old Welland Canal (heavily modified, regulated flow) then 12 Mile Creek	"fast and turbulent flows" (in 12 Mile Creek); ref, FF and NF flows of .5, 1 and .1 m/s	not reported	50% near-field sites; 1% or slightly greater at far-field sites	shorthead redhorse sucker *Moxostoma macrolepidotum*	shocking from boat (drifting down-stream) 3-6 Aug., 1994.	62 tot FF, 144 tot NF, 84 tot ref.	Males: NF longer & heavier livers than ref, FF heavier livers than ref, FF & ref heavier gonads than NF. Females: higher condition in FF than ref, heavier ovaries in ref than FF & NF, heavier livers in NF & FF than in ref	See ntbk for sample sizes at 3 diff sites. Only one fish sp. Abundant enough for use as sentinel sp. There are also a number of other industries and combined sewer overflow into river
St. Marys	mech.	1^o trt then	850 m^3/s	934 m^3/s	1% at 25m	white	3-4" gill	25 M, 25 F, 3	F: -length, +liver weight	1. yellow perch too

Paper, Sault Ste Marie, ON	Pulp mill producing 595 ADt/d	discharge to St Marys R.	avg., 350 m3/d low flow.	avg. (584-1126 m3/s)	down of diffuser	sucker	nets overnight, 13-19 Sept. 1995.	im, 84 tot ref; 26 M, 23 F, 1 im, 189 tot exp.	(at length), -body weight (outlier removed) M: +age, +liver weight, -gonad weight (outlier removed), body weight, length and condition SI	mobile? 2. exp fish collected outside 1% plume
	supercal-endered paper					yellow perch		15 M, 26 F, 43 tot ref; 3 M, 27 F, 1 im, 44 tot exp.	F: -age, -body weight, -length, +gonad weight, -egg volume, -fecundity	1. too few male perch for stats
Sonoco, Trenton ON, on Trent R.	tube- and linerboard from recycled materials	hydro-electric raceway, then lower perennial riverine system	not reported	2180 m3/d	not reported	silver redhorse suckers	gill nets (3,4,5" mesh) and electro-shock	25 M 22 F 3 IM ref; 20 M 20 F 0 IM exp (IM not inc)	F: +age, liver weight SI M: -age, + body weight (ref outlier removed), +liver weight	
						shorthead redhorse suckers	as above	22 M 23 F 2 IM ref; 22 M 24 F 1 IM exp. (IM not inc)	F: -body weight, -condition M: -body weight, -length, +liver weight, gonad weight	
Spruce Falls Inc., Kapuskasing, ON	producing 787 tonnes/d of newsprint	1º trt, then discharge to hydroelect. raceway, and Kapuskasing R.	0.1-1.5 m/s exp; negligible in ref.	0.855 m3/s	>1% in exp zone	white sucker	electrofishing day, gill nets (4" mesh) overnight	31 ref, 51 exp; 16-27 M and 15-24 F used for stats	F: +length, +condition, +liver weight, gonad weight SI M: +liver weight, -gonad weight SI	1. no stats on age or age distribution differences 2. difficult to get two sentinel spp., habitat of ref and exp zones "very different"(p.59)
						walleye	2-8 Oct. 1994	12 ref, 9 exp; 7-9 M and 2-3 F used in	too few for stats ; ref males older and bigger but exp males larger liver	Also no condition indices calculated no comments on walleye data
Strathcona Paper, Napanee ON	220 t/day coated boxboard (from recycled fibres)	2º trt (ASB) then facultative lagoons, then discharge	2-35 m³/s; lower perennial , sand and mud, 0.2-4 m deep (avg 2m deep)	2000 m3/d	1% at 15m down in high flow; 1.25% at 690m down at low flow	small-mouth bass	electrofishing from boat, 29 Aug.-2 Sept., 1995.	21 M, 23 F ref; 23 M, 23 F exp	F: -body weight, -gonad weight (at age) M: +age, -length, -body weight, +condition, +LSI, -gonad weight (at age)	-generally low power of stats in female groups; usually much higher in male groups -"external observations noted on field sheets" (p.3-7)

Appendix III

										but field sheets
		via diffuser to Napanee R.				rock bass		23 M, 24 F ref; 22 M, 23 F exp	F: +age, +LSI, condition SI M: -gonad weight	not included in report? -should sample later so female gonads fully developed?
Stone Consolidated Corp., Kenora ON, on Lake-of-the-Woods	groundwood and sulphite processes yield 860 tonnes/d newsprint and groundwood grade	1° trt then discharge into Winnipeg R. immediately below a dam at outflow of Lake (2° trt installed after	149 m3/s (1994 avg = 425 m^3/s); used past dye studies to calc flow during study	51921 m3/d (1995 avg = 48871 m3/d)	2% at 1200 m, 1% at 2500 m in low flow; avg flow, <1% within 300 m	white sucker	gill nets (2.5-4.5" mesh); 15 ref walleye donated by local fishers (giving away small ones?);	14 M, 25 F ref; 25 M, 23 F exp.	F: +liver weight, body weight, condition and gonad weight (at length) all SI M: +liver weight, +gonad weight (outliers removed)	Confounding: 1)STP outfall 2)use of lacustrine hab for exp sites but riverine for ref sites also too few walleye for stats
	paper	study completed)				walleye	22-29 Sept. 1995.	25 m, 20 f ref; 3 M, 0 F exp.	Not enough walleye to do stats	
Stone Consolidated, Fort Frances ON	BK pulp & paper, groundwood pulp; ClO$_2$	1° and 2° trt then river via diffuser	272-298 m^3/s in study; approx avg.	1994 avgs = 85-115 m3/s	<5.5% within 100m and vertically strat.; 1%	white sucker	gill nets (3-4" mesh) overnight, 20-30 Oct. 1994.	10 M, 9 F, 21 tot in ref; 24 M, 26 F, 57 tot exp	F: no stats M: no differences	other outfalls incl. Boise Cascade mill (127 000 m3/d), STP for 2 cities, many municipal
	bleaching used				and fully mixed by 600m	walleye		20 M, 7 F, 70 tot in ref; 22M, 6F, 46 tot in exp	F: no stats M: body weight and length SI	outfalls; also low numbers avail for females of both spp.
Thunder Bay Packaging Inc.	Thunder Bay, ON	directly to L. Superior; planned trt? Should be mentioned	not reported	predicted 2500 m^3/d	predicted 1% at 500 m south of outfall	spottail shiner	trap nets and seine nets; 7-25 Sept. 1995	79 at ref, 91 at exp; 20-21 used per sample for stats (how selected?)	F: -length, -condition, gonad weight SI M: condition and gonad weight SI	-only 1 sentinel sp available; also estimates of sample size are v. low, indicating. possible problems with using such young fish?

EEM CYCLE ONE FISH SURVEY REVIEW AND SUMMARY: WESTERN AND NORTHERN REGION

PART ONE: INTRODUCTION AND REGIONAL SUMMARY

Ten mills conducted Cycle One studies in the Western and Northern region. All discharge to freshwater (river or lake) environments.

Determining catch success was considered a key element of Cycle One and catch success for the Western and Northern region is presented in Table 13. Eight mills were successful for at least one species (*i.e.* captured at least 20 fish of each sex at a reference and an exposure site).

Table 13. Catch success for each Western and Northern region mill in EEM Cycle One. "Method" is the capture method used, where 1=single-mesh-size gill nets, 2=multi-mesh-size gill nets, 3=electroshocking, 4=seining, and 5=traps or trap nets.

Mill	**Species**		**Reference**		**Near-field**		
		Method	**Male**	**Female**	**Male**	**Female**	
Repap The Pas	white sucker	2	29	26	24	24	
	walleye	2	23	8	33	7	
Weyerhaeuser	white sucker	3	23	21	21	20	
Prince Albert SK	walleye	3	20	21	10	2	
ANC Whitecourt	lake chub	3	26	39	29	40	
	longnose sucker	3	9	20	18	19	
Daishowa Peace River	longnose sucker	3	20	21	19	20	
	burbot	3	12	13	19	22	
Pine Falls	white sucker	1	25	23	36	35	
	shorthead redhorse	1	16	13	20	16	
Weldwood	longnose sucker	3	30	45	7	15	
	mountain whitefish	3	20	22	20	23	
Slave Lake	white sucker	3	63	133	56	73	over 4 collection years
	longnose sucker	3					
Millar Western	lake chub	3	20	40	22	24	
	longnose sucker	3	18	19	19	21	
Alberta Pacific	walleye	3,4	6	3	6	1	
	longnose sucker	3,4	9	18	7	10	
Grande Prairie AB	mountain whitefish	3	30	45	39	22	
	longnose sucker	3	20	22	18	22	

Problems encountered and results observed are summarized in Tables 14 and 15 respectively, and discussed in detail on a mill-by-mill basis in Part Two of this report.

Table 14. Problems encountered by mills in Western and Northern region.

Problem	Number of Mills
Exposure not confirmed	3
Small gonads (immature or undeveloped)	1
Too few fish of one or both species	5
Plume small/deep/trapped/dissipated rapidly	1
Reporting unclear or incomplete	4
Only one sentinel species	0
Confounding factors	1
Reference site problems	4

Table 15. Results observed for female fish populations in Cycle One Fish Surveys at Western and Northern region mills (exposed relative to reference fish; 34 cases reported stats).

Parameter	Increase (%)	No difference (%)	Decrease (%)	Significant interaction
Age	0	10 (100)	0	na
Length at age	0	9 (90)	1 (10)	0
Weight at age	0	9 (90)	1 (10)	0
Gonad weight at body weight or length	0	8 (80)	2 (20)	0
Liver weight at body weight or length	1	9 (90)	0	0
Body weight at length	0	8 (80)	2 (20)	0
Fecundity	1	7 (70)	2 (20)	0

PART TWO: SUMMARIES AND REVIEWS OF CYCLE ONE STUDIES FOR WESTERN AND NORTHERN REGION MILLS

Mill: Alberta Newsprint Company, Whitecourt, AB

Problems:
1. Too few male longnose suckers (LNS) were captured at the reference site.
2. No power analyses were conducted on fish data, and no ANCOVAs on growth were reported.
3. No raw data were included in the report.
4. BKME discharge from a mill 175 km upstream (in Hinton AB) may be a confounding factor.
5. The exposure zone is very small.

Differences Observed:
Male LNS were older at the reference site.
Male and female lake chub from the reference site had higher condition factors than fish from the exposure site.
LSI was higher in exposed female LNS than in reference female LNS.

EEM Cycle 2 Considerations:

Mill: Daishowa-Marubeni International Ltd., Peace River, AB

Problems:
1. No power analyses were conducted on fish data, and no ANCOVAs on growth were reported.
2. No raw data were included in the report.
3. Burbot were not aged.

Differences Observed:
Energy storage differed between reference and exposure zones. Male longnose sucker from the exposure zone had heavier mean liver weight and greater LSI than male longnose suckers from the reference zone. Reference-zone male burbot had higher mean condition factor than exposed male burbot.

EEM Cycle 2 Considerations:

Mill: Millar Western Pulp Ltd., Whitecourt, AB

Problems:

1. Lake chub could not be aged.
2. Resin acid tracers in fish bile did not differentiate between exposure-zone and reference-zone fish, and three female longnose suckers which had been tagged in the exposure zone in fall 1993 were recaptured in the reference zone in this study (fall 1995).
3. No ANCOVA or power analysis results are reported. No raw data were included in the report.

Differences Observed:
Energy storage differed between sites. Exposed female lake chub had higher mean condition factor than reference female lake chub, and exposed male longnose sucker had higher mean condition factor than reference male longnose sucker.

EEM Cycle 2 Considerations:
Alberta Newsprint Company (ANC) is upstream of Millar Western Pulp (MWP) and their exposure zones overlap. Since the area between the ANC and MWP diffusers does not appear to be a suitable reference site for MWP (fish may move back and forth into MWP exposure zone), ANC and MWP should consider combining their adult fish surveys for Cycle 2. The low effluent concentration in exposure zone and limited size of the exposure zone should be considered in designing Cycle 2.

Mill: Pine Falls Paper Co., Pine Falls, MB

Problems:

1. Too few shorthead redhorse sucker were captured.
2. The exposure-zone collection site is not always within the 1% effluent plume.
3. No total catch information is reported (*i.e.* other species caught in nets).
4. The results state that "use of a 4.0 inch gill net had an apparent size selection effect on the fish captured, as observed in the data", but there is no mention in the methods or elsewhere of where or why this net was used (methodology states that 3.5 inch monofilament gill nets were used for fish collection).
5. Neither the results of RFA tracer analysis nor the results of water parameters measured during the fish collections seem to be included in the report.
6. No reference was made in the report to data from government and non-government studies conducted in the past or concurrently with the Cycle 1 work.

Differences Observed:
Length at age differed for male white suckers from the reference and exposure zones.
Liver weight (at body weight) showed significant interaction in male and female white suckers.
Gonad weight (at body weight) was heavier in female and male white suckers from the reference zone.
Fecundity (at body weight) was greater in female shorthead redhorse sucker from the reference zone.

EEM Cycle 2 Considerations:

Mill: Repap Manitoba, The Pas, MB

Problems:

1. Too few walleye.
2. Some female fish may have been immature.

Differences Observed:
There were no statistics for female walleye (too few fish), and female white sucker showed no significant differences.
Exposed male white suckers (WhS) were younger than reference, and exposed male walleye (WALL) were older than reference fish.
Growth of male WhS showed significant interaction.

Exposed male WHS showed decreased condition, while exposed male WALL showed increased liver weight (at body weight).
Gonad weight was decreased in exposed male WHS and showed significant interaction in male WALL.

EEM Cycle 2 Considerations:

Mill: **Slave Lake Pulp Corporation, Slave Lake, AB**

Problems:

1. Too few longnose sucker were collected from the reference zone.
2. This site presents unique habitat considerations, as upstream and downstream habitats differ dramatically.

Differences Observed:
Some differences observed in female white sucker before mill start-up; disappeared or reversed after start-up.

EEM Cycle 2 Considerations:
A unique baseline data set exists for this mill and should be continued. This data set may be used instead of a reference site for longnose and white sucker studies in future cycles of EEM.
The report suggests spottail shiners or an invertebrate such as *Hyalella azteca* or larval *Ephemeroptera* or *Odonata* be used as sentinel species in Cycle 2. Since spottail shiners are abundant in reference and exposure areas they may be investigated as the second sentinel species in Cycle 2.

Mill: **Weldwood of Canada Limited, Hinton, AB**

Problems:

1. Too few longnose sucker were obtained from the exposure zone.
2. No details of the habitat in either zone are provided in the report (possibly contained in the design document).
3. No condition factor, GSI or LSI are reported.
4. Migration of fish in the river may confound interpretation of data.
5. The Athabasca R. is an open system therefore there may be problems with exposure of the fish.

Differences Observed:
Results of ANOVAs are not reported (only power analyses results are given). The means and standard deviations indicate the following trends, which may or may not be statistically significant:
Males and females of both species were older in the reference zone. Male and female mountain whitefish were longer and heavier and had heavier gonads in the reference zone but heavier livers in the exposure zone. For both species, variability was greater in the reference zone (liver weight was the main exception). Mean egg diameter was greater in exposed females of both species.

EEM Cycle 2 Considerations:
Spoonhead sculpin and other forage species should be considered for use as sentinel species in Cycle 2. Use may be made of historical data on fish within the receiving environment as reference fish rather than using a reference site located in a separate river system.

Mill: **Weyerhaeuser Canada Ltd., Grande Prairie, AB**

Problems:

1. Results of ANCOVAs were not reported, so statistical significance of the apparent trends in the data is not known.

2. No indices were reported (GSI, LSI, condition factor).
3. Reference area is located on a different river system (because of the documented mobility of the two sentinel species, longnose sucker and mountain whitefish).

Differences Observed:
No statistical tests of differences were reported but the means and standard deviations of the samples indicate the following:
Longnose sucker males and females appear to be older, longer and heavier in the reference zone, with heavier gonads but similar liver weights. Standard deviations overlap on gonad weights.
Mountain whitefish means and standard deviations are similar for age and body measurements in the two zones.

EEM Cycle 2 Considerations:
This mill has exceptionally good baseline data for their receiving environment and this may be used instead of a reference site in future EEM cycles.

Mill: Weyerhaeuser Saskatchewan Ltd., Prince Albert, SK

Problems:

1. Fish were sampled during the spawning season. Since both sentinel species (white sucker and walleye) migrate some distance to spawning grounds, fish sampled from the exposure zone may not be exposed to mill effluent for more than the spawning period. Thus the long-term effects of effluent exposure on growth and reproductive parameters cannot be determined from this data. Furthermore chemical tracers were unsuccessful in confirming exposure/non-exposure of fish.
2. Results of ANCOVAs were not reported, so statistical significance of the apparent trends in the data is not known.
3. Too few mature walleye (10 M, 2 F) were captured in the exposure zone, and the local walleye fishery confounds interpretation of data on this species.
4. Gonad weight was determined from "gonadal material (actual organ and any sexual product extruded earlier)".
5. Reference and exposure sites were located on different river systems.

Differences Observed:
No ANCOVA results were reported. Means and standard deviations indicate that white suckers from the exposure zone are younger, larger and heavier than in the reference area, but with lighter gonads and slightly heavier livers. Walleye from the exposure zone are also younger and heavier but with lighter gonads (but small sample size in exposure zone).

EEM Cycle 2 Considerations:
Use of a reference site on a separate river system did not allow useful comparison of data. A reference site located within the North Saskatchewan River system should be considered. Fish should not be collected during spawning season. Walleye should not be considered as a sentinel species (as recommended by the Saskatchewan TAP which rejected walleye as a sentinel species in the Weyerhaeuser Study Design). Instead, resident populations of forage fish may be considered to replace walleye as a sentinel species.

Table 16 summarizes the data on each mill and the details of the Fish Survey as provided in the Cycle One report. Mills are listed in alphabetical order. "Differences in exposed fish relative to reference" are for statistically significant differences only, unless otherwise noted.

Table 16. Summary of Fish Survey designs and results for Western and Northern region mills.

Mill & Location	**Type of Mill**	**Discharge environment**	**Receiver flow**	**Effluent flow**	**Approx. effluent conc.**	**Sentinel species**	**Collection method, dates**	**Numbers collected**	**Differences in exposed fish relative to reference**	**Problems**
Alberta Newsprint Company, Whitecourt, AB	TMP, DIP and paper mill, up to 620 t/d	2^0 trt then discharge to Athabasca R.	50.2-655 m^3/s; 334 m^3/s annual mean	16 000 m^3/d (effluent and cooling water)	<1% at 100m at lowest flow	lake chub	shocking (boat and backpack) and seining	26 M, 39 F ref; 29 M, 40 F exp	F: -condition M: -condition	- BKME discharge 175 km up, at Hinton, into Athabasca R. -no aging of chub -no raw data reported
						longnose sucker	9 Sept - 22 Oct, 1995	9 M, 20 F ref; 18 M, 19 F exp.	F: +LSI M: -age	-sample sizes were not always met -no power analysis
Alberta Pacific, Boyle AB		Athabasca R.	193-215 m^3/s		1% to 2km down-stream	walleye	shocking, seines, set lines; 8-19 Oct 1995	6 M, 3 F ref; 6 M, 1 F exp.	No stats	-too few fish, could use minnows -no suitable tracer -exposure area larger than 1% plume
						longnose sucker		9 M, 18 F ref; 7M, 10 f exp.	F: + liver weight, +LSI	
Daishowa-Marubeni International Ltd., Peace River, AB	BKM, 50-70% ClO_2 sub., 1272 ADt/d	2^0 trt (ASB) then discharge to Peace R.	~ 1500 m^3/s	not reported	<1% in 175 m; <0.05% in exp, low flow	longnose sucker	shocking (boat) and set lines overnight	20 M, 21 F ref; 19 M, 20 F exp	M: +liver weight, +LSI	-no ANCOVAs or power analyses reported -no aging of burbot
						burbot	26 Oct - 20 Nov 1994	12 M, 13 F ref; 19 M, 22 F exp.	M: -condition	
Millar Western Pulp Ltd., Whitecourt, AB	CTMP, 620 t/d alkaline peroxide pulp	2^0 trt then discharge to Athabasca R.	119-164 m^3/s	11 000 m^3/d	0.03-0.75% in exp zone	lake chub	boat and backpack shocking, seining	20 M, 40 F ref; 22 M, 24 F exp.	F: +condition	-chub not aged -tracers inconclusive
						longnose	Sept-Oct	18 M, 19 F	M: +condition	

						sucker	1995	ref; 19 M, 21 F exp.		
Pine Falls Paper Co., Pine Falls, MB	ground-wood and sulphite pulp mill;	1^0 trt then dishcarge to Winnipeg R.	not reported	approx. 25000 m3/d	not reported	white suckers	3.5-4" gill nets overnight; 25 Aug. - 25 Sept., 1995.	25 M, 23 F ref; 36 M, 35 F exp.	F: -gonad weight, liver weight SI M: -gonad weight, liver weight SI, length differed	-ANCOVAs not summarized -exposure site outside 1% effluent plume at low and mean flows of the Winnipeg R.
	175000 Adt/d of news-print					short-head redhorse		16 M, 13 F ref; 20 M, 16 F exp.	F: -fecundity M: no differences	
Repap Manitoba, The Pas, MB	400 Adt/d unbleached kraft paper	2^0 trt then open channel to Saskatchewan R. (lower perennial)	not reported	not reported	1% in 2-3 km depending on flow	white sucker	3.5-4.5" gill nets	29 M, 26 F ref; 24 M, 24 F exp.	F: no differences M: -age, growth SI, -condition, -gonad weight	-some sucker females immature? see gonad weights
						walleye	26 Sept-3 Oct, 1995.	23 M, 8 F ref; 33 M, 7 F exp.	F: no stats M: +age, +liver weight, gonad weight SI	-too few female walleye for statistics
Slave Lake Pulp Corporation, Slave Lake AB	BCTMP mill; 350 ADt/d non-Cl bleaching	2^0 trt (biological) then discharge to Lesser Slave River	34.3 m^3/s mean; 18.9 m^3/s low mean, 54.2 m^3/s peak mean	43.6 m^3/s (1994 mean)	<1% at 500 m from diffuser	white sucker	shocking from boat; mid-Sept, 1989-94	63 M, 133 F ref; 56 M, 73 F exp; over the four years of collection	F: -length, -condition, -gonad weigth (at length), -fecundity **before** mill start-up; then differences disappeared or reversed; M: no differences before or after	-too few LNS; need an alternate sentinel sp.
						longnose sucker	as above			
Weldwood of Canada Ltd., Hinton, AB	100% ClO$_2$ sub	not reported	not reported	not reported	not reported	longnose sucker	shocking from boat, daylight 29 Apr - 12 May	30 M, 45 F ref; 7 M, 15 F exp.	F: no differences M: liver weight SI	-ANCOVA results not reported; power analyses only -no details of habitat or
						mountain whitefish	shocking from boat, daylight 23 Aug - 5	20 M, 22 F ref; 20 M, 23 F exp.	F: no differences M: body weight, length, condition, liver weight SI	of shocking in either zone -too few LNS from exposure zone

							Sept 1995			
Weyerhaeuser Canada, Grande Prairie AB	chlorine bleaching ceased June 1992	not reported	not reported	not reported	not reported	mountain whitefish	shocking from boat; 5-20 May 1994	30 M, 45 F ref; 39 M, 22 F exp.	no stats but means indicate exposed fish are smaller, younger, lighet body and gonads and heavier liver; exposed females fewer eggs	-tracers did not confirm exposure -no ANCOVAs or GSI, LSI reported
						longnose sucker	shocking from boat; 23 Aug-10 Sept 1994	20 M, 22 F ref; 18 M, 22 F exp.	no stats but exposed fish larger, heavier body and organs, exposed females older	
Weyerhaeuser Saskatchewan Ltd., Prince Albert, SK	BK mill	Saskatchewan R.	not reported	not reported	1% at 250-4000 m down	white sucker	trap net at ref; electro-shock at both	21 M, 20F exp; 23 M, 21 F ref.	-no ANCOVA results shown, but exposed fish are younger, larger and heavier but with lighter gonads and slightly heavier livers; similar trends when outliers removed	-both spp. sampled during spawning season -reference site is in a different river
						walleye	23 Apr - 7 May, 1992	10 M, 2 F exp; 20 M, 21F ref	-no ANCOVA results; trends not obvious but exposed fish were younger & heavier but smaller gonads	-few supporting data for reference zone -different capture methods at reference and exposure sites

EEM CYCLE ONE FISH SURVEY REVIEW AND SUMMARY: PACIFIC AND YUKON REGION

PART ONE: INTRODUCTION AND REGIONAL SUMMARY

Twenty three mills conducted studies for Cycle One in the Pacific and Yukon region. Thirteen were for mills that discharge to marine/estuarine environments and ten were for mills that discharge to freshwater (river or lake) environments.

Determining catch success was considered a key element of Cycle One and catch success for the Pacific and Yukon region is presented in Table 17. Eight marine/estuarine mills (9 if include all-juvenile starry flounder catch at Scott) and five freshwater mills were successful for at least one species (*i.e.* captured at least 20 fish of each sex at a reference and an exposure site).

Table 17. Catch success for each Pacific and Yukon region mill in EEM Cycle One. "Method" is the capture method used, where 1=single-mesh-size gill nets, 2=multi-mesh-size gill nets, 3=electroshocking, 4=seining, 5=traps or trap nets, 6=hand picking, 7= other (e.g. longlines) and 8=trawling.

Mill	**Species**	**Method**	**Reference**		**Near-field**		**Far-field**	
			Male	**Female**	**Male**	**Female**	**Male**	**Female**
MARINE:								
Alberni	Threespine sickleback	4	20	20	20	20	20	20
	English sole	8	18	20	20	20		
Two reference areas for Threespine (Nahmint Bay and Effingham Inlet). One for English sole (Barkley Sound).								
FCC Crofton	English sole	8	15	28	11	15		
	Pacific oyster	6	42		63		61	
	Dungeness crab	5	0	0	32	1	54	1
FCC Elk Falls	Quillback rockfish	7	12	10	28	14		
	Dungeness crab	5	37	1	41	2	106	36
Eurocan Kitimat	Starry flounder	4,5	76		140			
	Prickly sculpin	4,5	43, 31	20, 7	20	21	Two reference areas	
	Staghorn sculpin	4,5	10	0	4	20		
Avenor Gold River	Widow rockfish	2	11	11	51	34		
	Red rock crab	5	3	0	8	3		
Harmac Nanaimo	English sole	8	21	24	41	20	27	24
	Dungeness crab	5	36	4	23	17	35	5
	Two spot prawn	8	28	1	7	10	21	5
Paperboard	Prickly sculpin		22	20	21	26		
	Staghorn sculpin		10	11	10	14		
	Starry flounder		46 Juveniles		40 Juveniles			
Port Alice	Slender sole	7,8	0	0	20	20		
	Blue mussels	6	63		63			
	Dungeness crab	5	23	20	12	27		
Port Mellon	Dungeness crab	5	31	6	42	13		
	Blue mussels	6	30		30			
MacBlo Powell River	English sole	8	1	1	13	16		
	Pacific oyster	6	42		84		42	
	Dungeness crab	5	2 - not dissected		19	4	10	0
Scott	Starry flounder	4,5	97		53		69	

	Prickly sculpin	4,5	14	6	15	2	4	7
	Prickly sculpin	4,5	48 Juveniles		39 Juveniles		29 Juveniles	
Skeena	Rock sole	8	7	7	7	5	119	33
	Dungeness crab	5	55	32	73	5		
Woodfibre	Dungeness crab	5	4	0	42	7		
	Blue mussels	6	30		30			
FRESHWATER:								
Canfor	Largescale sucker	3,2,4	24	23	24	22		
	Peamouth chub	3,2,4	25	27	20	20		
Cariboo	Largescale sucker	3,2,4	25	23	22	20		
	Peamouth chub	3,2,4	20	27	12	14		
Celgar	Mountain whitefish	3,2	17	15	11	10		
	Longnose sucker	3,2	1 - not dissected		21	20		
Crestbrook	Mountain whitefish	3	21	21	21	21		
	Largescale sucker	3	7	13	8	19		
Fibreco	Mountain whitefish	3	20	20	20	20	20	20
	Longnose sucker	3	20	20	20	20	20	20
Finlay	Lake whitefish	2	23	22	19	29	9	26
	Longnose sucker	2	30	10	14	13	2	2
	Largescale sucker	2	3	0	11	10	1	26
Mackenzie	Lake whitefish	2	Same fish as FINLAY					
	Longnose/Largescale sucker	2						
Northwood	Largescale sucker	3,2,4	Same fish as CANFOR					
	Peamouth chub	3,2,4						
Quesnel	Largescale sucker	3,2,4	Same fish as CARIBOO					
	Peamouth chub	3,2,4						
Weyerhaeuser	Largescale sucker	3,2	21	21	22	21		
	Peamouth chub	3,2	21	21	17	30		

Starry flounder, juvenile Prickly sculpin, mussels, and oysters were not sexed

Problems encountered and results observed are summarized in Tables 18 and 19 respectively, and discussed in detail on a mill-by-mill basis in Part Two of this report.

Table 18. Problems encountered by mills in Pacific and Yukon region.

Problem	Number of Mills
Exposure not confirmed	4
Small gonads (immature or undeveloped)	9
Too few fish of one or both species	10
Plume small/deep/trapped/dissipated rapidly	7
Reporting unclear or incomplete	6
Only one sentinel species	1
Confounding factors	7
Reference site problems	4

Table 19. Results observed for female fish populations in Cycle One Fish Surveys at Pacific and Yukon region mills (exposed relative to reference fish; 11 cases included here).

Parameter	Increase (%)	No difference (%)	Decrease (%)	Significant interaction

Age	1 (9)	4 (36)	6 (54)	na
Length at age	2 (18)	7 (63)	2 (18)	0
Weight at age	0	8 (73)	3 (27)	0
Gonad weight at body weight or length	0	10 (90)	1 (9)	0
Liver weight at body weight or length	0	9 (81)	2 (18)	0
Body weight at length	4 (36)	4 (36)	3 (27)	0
Fecundity	0	11 (100)	0	0

PART TWO: SUMMARIES AND REVIEWS OF CYCLE ONE STUDIES FOR PACIFIC AND YUKON REGION MILLS

Mill: Avenor Inc., Gold River BC

Problems:

1. Only one sentinel species was available, and only 11 of each sex were captured in the reference zone. It was the widow rockfish, which is slow-growing and not an ideal EEM sentinel species.
2. Fish ages were not reported because the difficulty of aging these fish resulted in highly variable and unreliable age data. For this reason no statistics on growth parameters were conducted. Nonetheless, it would have been appropriate to report the age data with the fish summary statistics.
3. Few fish had gonads weighing more than 1g, so no statisticss on gonads were conducted.
4. Reference fish had higher standard and maximum TEQ values for the dioxins/furans used as a tracer in fish tissue than the exposed fish. This does not confirm that the reference fish were not exposed to effluents of some kind.

Differences Observed:
Exposed male widow rockfish had heavier livers (at length) when the entire data set was used. When larger fish were removed from the exposure zone sample so that only fish of a similar size range were used from both sites, the difference in liver weight at length was not significant.

EEM Cycle 2 Considerations:
The widow rockfish is not an appropriate sentinel species. An alternative to the fish survey may be appropriate for this site.

Mill: Celgar Pulp Company, Castlegar, BC

Problems:

1. Only one sentinel species (mountain whitefish, MWF) was available in the reference zone. Longnose sucker were readily captured in the exposure zone but only one specimen was caught in the reference zone.
2. Some immature MWF were captured (maturity class 2) and included in the table of summary statistics with gonad weights as low as 0.1 g and GSIs as low as 0.06. (There are more immature fish at the exposure site than the reference site and this could affect the outcome of ANCOVAs.)
3. Reference zone habitat was classified as pools while exposure habitat was classified as glide.

Differences Observed:
Growth differed between sites with female MWF longer (at age) in the exposure zone. Male and female MWF were younger and heavier (at length) in the exposure zone.
Gonads were heavier (at body weight) in exposed male MWF than references males, and exposed females had higher egg volume (at body weight) than reference females.

The health assessment index was highly variable. There was no significant difference in HAI between reference and exposed fish.
No longnose suckers were caught in the reference zone so there is no comparison; however complete data were recorded for longnose suckers collected from the exposure zone for use in future EEM cycles.

EEM Cycle 2 Considerations:
Mountain whitefish and longnose sucker are suitable sentinel species but effort must be made to catch longnose suckers in a reference area. Different reference areas may be needed for different sentinel species, as recommended in the report. Immature fish should not be included in analyses of variance.

Mill: Crown Packaging Inc.(Paperboard), Burnaby, BC

Problems:
1. All flounder were juvenile and no fish had well-developed gonads.
2. No data for age were reported. Regressions of weight and length against age were used to determine which was the best indicator of age.
3. Too few fish were collected for the prickly and staghorn sculpin samples.
4. There are a number of confounding discharges in the area (however the study was designed to test for differences due to Crown Packaging effluent only and the placement of the reference site has avoided most confounding dishcarges).

Differences Observed:
Weight (at age) of male and female prickly sculpins differed between reference and exposed sites.
Gonad weight (at standard length) differed for female staghorn sculpins from reference and exposed sites.
There were no other significant differences.

EEM Cycle 2 Considerations:
Other discharges, small 1% field and high dilution are confounding factors. An alternative survey at this location is appropriate.

Mill: Crestbrook Forest Industries Ltd., Cranbrook, BC

Problems:
1. Too few largescale sucker were collected (20 at the reference site, 27 at the exposure site) because spawning kokanee interfered with electrofishing.
2. Most mountain whitefish were given a maturity rating of 7 (*i.e.* gonads are "spent or recovering"; only 5 females in total had eggs), therefore gonad weights do not reflect true allocation to reproduction.
3. Dioxin, furan and TEQ levels in largescale suckers decrease with distance from the diffuser and are lowest in the reference area fish; however this is not the case with mountain whitefish, which showed varying levels of these contaminants throughout the study region.

Differences Observed:
Mountain whitefish (MWF) were older in the reference zone.
Growth differed for female MWF (significant interaction for length-at-age).
Gonads weight (at body weight) was greater in reference male MWF than exposed male MWF. This was also true for female MWF when gravid females were removed from the analysis.
Health assessment indices were highly variable but showed no significant differences overall between reference and exposed sites for either sentinel species.

No ANOVAs or ANCOVAs were conducted for largescale suckers (LSS), but summary stats and plots indicate that LSS of both sexes were larger in the exposure zone, and the exposed males are heavier (at age) and have a greater GSI than reference males.

EEM Cycle 2 Considerations:
Effort should be made to collect sufficient numbers of largescale suckers. The timing of sample collections should be reviewed with respect to the spawning period of mountain whitefish and of kokanee (to facilitate capture of largescale suckers). Investigations into movements of mountain whitefish would help to determine consistency of exposure of these fish. They may need to be sampled elsewhere. Sharing of regional reference sites with the Celgar mill at Castlegar, BC should be considered.

Mill: Crofton Pulp and Paper (Fletcher Challenge Canada), Crofton, BC

Problems:
1. Too few English sole were collected from both reference and exposure sites. Many of the sole collected were sexually immature or had undeveloped gonads.
2. Collection methods for oysters may lead to selection for a particular size range (collectors were asked to collect "a variety of sizes").
3. No ANCOVAs or power analyses were reported for the data. Graphs of various traits are provided for comparison, and they are designed to compare sexes (of English sole) at the same site, rather than comparing a single sex among sites as they should do.
4. No crabs were collected from the reference zone so there is no comparison of organochlorine levels between exposed and reference zones, only between historical and current exposure-zone data.

Differences Observed:
No statistics.
Oysters appear to be more abundant and larger in the near-field zone, but in better condition in the reference zone.

EEM Cycle 2 Considerations:
Sampling time may have to be later in the year to allow maturation of gonads in both flounder and oysters. A new sentinel species should be sought to replace English sole. Trapped plume and smalll 1%field limit fishing; an alternative to the fish survey may be appropriate for this mill.

Mill: Elk Falls Pulp & Paper (FCC Ltd.), Campbell River, BC

Problems:
1. Too few quillback rockfish were collected. Many rockfish had undeveloped gonads.
2. No information was reported on gonads of Dungeness crabs.
3. Swift currents cause rapid dilution of effluent.

Differences Observed:
Age: Exposed male rockfish were younger than reference male rockfish and the age distributions for male rockfish differed significantly.
Condition factor (ANCOVA of weight with length as covariate) was greater in exposed female rockfish than in reference females.
Hepatopancreas weight as a percentage of body weight and was greater in near-field male crabs than in reference males.
Correspondance analyses indicate that the exposure site has a weak correlation with fouled gills and with moderate numbers of external lesions for crabs.

EEM Cycle 2 Considerations:
Fast currents and a rapidly diffused effluent result in a small exposure zone and are reasons to seek alternatives for this site.

Mill: Eurocan Pulp and Paper Ltd., Kitimat, BC

Problems:

1. Several different industrial discharges in the area may be confounding factors.
2. Too few fish were collected from some sites, usually from reference site 2. For both starry flounder and staghorn sculpin, fish were too young and gonads were very small (not measurable in flounder).
3. Reference site 2 was too close to the exposure site.

Differences Observed:
Weight-at-age, condition and liver weight all differed significantly among various sites, for males and females.
There was significant interaction for gonad weight of male and female prickly sculpins.

EEM Cycle 2 Considerations:
A larger scale survey may be appropriate here, to help deal with the many industrial inputs which confound the survey.

Mill: Fibreco Pulp, Taylor, BC

Problems:
No total catch data, summary statistics or indices (GSI, LSI) were included, although they were listed in the Table of Contents for the Appendices.

Differences Observed:
Female mountain whitefish (MWF) were oldest in the near-field (NF) area. Reference females were youngest and average age of far-field (FF) fish was in between. Female longnose sucker (LNS) were oldest in the reference area, and youngest in the NF, with FF in between.
Growth: Standard length (at age) of female MWF was greatest in the FF zone and least in the reference zone. For male and female LNS, standard length (at age) was greatest in the NF zone and approximately equal in the FF and reference zones. Condition (body weight at length) showed a similar trend in three groups, with female MWF heaviest (at length) in the reference zone and lightest in the FF, and LNS of both sexes heaviest (at length) in the reference zone but lightest in the NF.
Liver weight at body weight showed significant differences in MWF males and LNS females. Male MWF had heavier livers (at body weight) in the reference and FF zones than in the NF. Female LNS had heavier livers (at body weight) in the NF and reference zones than in the FF. Female MWF showed significant interaction of liver weight at body weight.
Although not significantly different, gonad weight (at body weight) was decreased by 13-27% in NF fish of both sexes and species, relative to reference fish.

EEM Cycle 2 Considerations:
Although there are differences between reference and nearfield fish, the large dilution and small 1% field limit exposure. The fish survey could continue with small-bodied fish.

Mill: Harmac Pacific Inc., Nanaimo, BC

Problems:

1. ANOVAs on length and weight were conducted on combined male and female data for both sentinel species (although there are sufficient numbers of each sex for separate analyses).
2. The "power chart" used to report ANCOVA outcomes is confusing and poorly copied (difficult to read), and p values are not shown on that table. Some p values are given in the text but the writing is sometimes ambivalent. The ANCOVAs for each species should be summarized clearly and with p values included in one table.
3. The chlorinated phenols used as tracers were not successful in demonstrating exposure/non-exposure of fish from near-field, far-field and reference areas.
4. Many fish had gonad weights of less than one gram.
5. GSI and LSI were not reported.
6. There is a small 1% field and trapped plume.

Differences Observed:

Age differed for English sole, both sexes of which were older in the far-field zone.
Condition (body weight-at-length)was greater in near-field and far-field sole of both sexes than in reference sole. The opposite is true for female crabs, which were heavier in the reference and far-field areas than in the near-field area.
Liver weight (at body weight) showed significant interaction in female sole.
Gonads were heavier in male crabs from the reference zone than from the near-field.

EEM Cycle 2 Considerations:

Another tracer should be examined in fish tissue to confirm exposure/non-exposure of the different samples. English sole are not an appropriate sentinel species. An alternative to the fish survey may be required to deal with the small trapped plume at this site.

Mill: Howe Sound (Western Pulp Limited Partnership, [WPLP] Squamish and Howe Sound Pulp & Paper Limited, [HSP&P] Port Mellon)

Problems:

1. Too few English sole (total N=3) and very few finfish in general were captured.
2. No ANCOVAs or power analyses conducted on the other two, more abundant sentinel species (Dungeness crab and blue mussel).
3. Collections in the exposure zones were done in March 1995 and collections in the reference zone were in May 1995.
4. Most of the blue mussels from exposure sites were too small to obtain an accurate measure of dry weight and were therefore excluded from the condition index calculations. Comparisons of condition indices between reference and exposure sites therefore cannot be made.
5. Collection of mussels could be biased (hand picking for a range of sizes) and confounds comparison among sites because a range of sizes is collected, rather than a sample of similar-sized mussels.
6. The effluent plume is trapped at 15-50 m.

Differences Observed:

The following are not statistically tested differences since no ANOVAs or ANCOVAs were reported for these data.
Blue mussels from the exposure zone were smaller and had lower condition indices than those from the reference zone (but see above, #4).
The mean values for hepatopancreas weight as percentage of body weight are higher in the near-field zones than in the reference zones (for Dungeness crab).

EEM Cycle 2 Considerations:

A new reference area should be sought because Toba Inlet does not appear to be appropriate. An alternative to the fish survey may be needed due to the trapped plume.

Mill: **Macmillan Bloedel, Alberni Pulp and Paper, Port Alberni, BC**

Problems:

1. The report is incomplete. Data are reported as graphs only, and sample sizes for different sexes are not given. Each graph includes data for both sexes from one site only, which makes comparisons of each sex by site especially difficult
2. Too few English sole were collected from the reference areas.
3. No suitable tracer was found. Colour in water was used as a tracer but did not confirm exposure of sentinel species (colour <5 in exposure areas, but 138-209 in effluent; not measured in reference areas).
4. Many sole had gonads less than 2 g in weight, indicating that they may not have been fully developed.

Differences Observed:

No conclusions can be reached from the data as presented in this report.

No age data or graphed comparisons for sticklebacks.

EEM Cycle 2 Considerations:

Too few sole were caught in the reference zones, but catch data indicate that several other types of fish are abundant and may be more suitable for the Cycle 2 AFS (*e.g.* sculpin, slender sole, eelpout). There are a number of confounding factors (other inputs to Alberni Harbour) which should be considered.

Mill: **Macmillan-Bloedel Powell River Div., Powell River, BC**

Problems:

1. Collections in the exposure zones were done in March 1995 and collections in the reference zone were in May 1995. Trawling depths differed between the two zones (>50m in reference, >100m in exposure).
2. Too few English sole were collected from the exposure zone.
3. Collection of oysters could be biased (hand picking for a range of sizes) and confounds comparison among sites because a range of sizes is collected, rather than a sample of similar-sized oysters.
4. There are no statistics (ANOVA or power analyses) for oysters even though sample sizes were sufficient.
5. Effluent plume is trapped at 15-50 m.

Differences Observed:

No firm conclusions can be reached because (1) too few of one sentinel species (sole) were collected, (2) no statistics (ANOVAs) were reported for the second sentinel species (Pacific oyster), and (3) the timing of the collections makes the observed differences in condition factor of oysters less meaningful.

EEM Cycle 2 Considerations:

Collections from reference and exposed sites must occur at the same time of year. An alternative sentinel species is required to replace English sole. An alternative reference site may be required for fish if Qualicum Beach does not offer deep enough habitat to compare with the exposed site. An alternative to the fish survey may be needed due to the trapped plume.

Mill: **Repap British Columbia Inc. (Skeena), Prince Rupert, BC**

Problems:

1. Too few rock sole were collected from the both zones.
2. Size and age ranges of sole from reference and exposure areas differed, possibly because of different trawling depths at the two sites. This may also explain the different numbers of invertebrates captured by trawling in the two areas (*e.g.* over 8000 coonstripe shrimp in the exposed area and 500 in the reference area).
3. No graphs provided for crab data.
4. A total of 150 blue mussels were collected as an alternative sentinel species but are never mentioned in the results. Since the required numbers of rock sole were not collected, the blue mussel data should have been reported as a second sentinel species along with Dungeness crab.
5. No data on crab gonads were reported.
6. Small 1% field.

Differences Observed:

No statistical tests were done on the rock sole data because there were too few fish, but the rock sole sample from the reference site included larger, older individuals (up to 15 years old) than the exposed-area sample (up to 6 years old).

Correspondance analyses showed that near-field male crabs were heavier in relation to carapace width and had heavier hepatopancreas than reference male crabs.

EEM Cycle 2 Considerations:

A second sentinel species should be collected in sufficient numbers for statistical analyses. Fishing effort for finfish consisted of four days of trawling (3 in the exposed area, 1 in the reference area; a total of <3.5 hours per zone). This effort could be increased if necessary to obtain the required number of specimens. Otherwise, blue mussels could replace rock sole as a sentinel species. An alternative may be required if exposure cannot be demonstrated (due to small plume).

Mill: **Scott Paper Limited, New Westminster, BC**

Problems:

1. Very few adult fish were collected (total of 15 female and 33 male prickly sculpin from all sites). Statistical analyses of adult male and female starry flounder are referred to in the text but no sample size is given and no tables mention any adults of this species.
2. Gonads are very small in adult prickly sculpin (means range from 0.03-0.45 g; GSI<1%).
3. Reference sites were combined for statistical analyses but it is unclear whether exposure sites were combined. This should be clarified because only the near-field exposure site was within the 1% effluent plume.
4. The reference sites are close to the exposure sites (across the river).
5. There are confounding discharges and dredging activity in the area.

Differences Observed:

Body weight at length showed significant interaction for juvenile flounder. Juvenile prickly sculpin had significantly different body weight (at length).

Liver weight (at body weight) was significantly different for female sculpins.

Gonad weight showed significant interaction for juvenile prickly sculpin.

EEM Cycle 2 Considerations:

An alternative may be required due to the many confounding inputs to this system.

Mill: **Upper Fraser River - 4 Mill Study (Northwood Pulp and Timber Ltd., Prince George; Canadian Forest Products Ltd., Prince George; Quesnel River Pulp Company, Quesnel; Cariboo Pulp & Paper Company, Quesnel, BC)**

Problems:

1. Most fish of both sentinel species (largescale sucker, LSS and peamouth chub, PMC) had undeveloped gonads.
2. No tracer was used in fish. Dioxin/furan content of mountain whitefish was measured at downstream sites but used a different reference site than the AFS reference site.
3. Too few PMC were captured at the Quesnel exposure site.

Differences Observed:

Age differed significantly among sites for both species. For LSS, females from the Woodpecker (far-field) and Quesnel (near-field) sites were significantly older than those from the reference site and Pr. George (near-field) site. LSS males were significantly older at Woodpecker (far-field) than at Pr. George (near-field), but no other sites were different from each other. For PMC, females were older at the reference and Pr. George (near-field) sites (opposite to LSS females). Ages of PMC males did not differ among sites.

Size (length-at-age) was significantly different for LSS males and PMC males and females. In all three cases, fish were largest (at age) at the Pr. George site (near-field), and the males of both species were shortest at the Quesnel (near-field) site. Female LSS showed significant interaction in length-at-age.

Liver weight (at body weight) was highest at Pr. George (near-field) for LSS females.

Gonad weight (at body weight) showed significant interaction for male and female LSS and male PMC. Female PMC had heavier gonads at the reference and Pr. George (near-field) sites than at the Woodpecker (far-field) and Quesnel (near-field) sites.

Condition factor did not differ significantly between sexes or among sites for either species.

EEM Cycle 2 Considerations:

The discussion states that electrofishing was non-selective and that many fish were missed because of turbid water. Gill nets may be more efficient and selective at catching the larger fish needed in order to examine fully developed gonads and should be considered as an additional collection method in Cycle 2.

Mill: Western Pulp Limited Partnership (Port Alice), Port Alice, BC

Problems:

1. No slender sole were captured in the reference zone. Sufficient numbers of blue mussels were collected as the second sentinel species but no statistical analyses were reported.
2. No stats reported in Dungeness crab for this mill alone; the overall report on Dungeness crab on Howe Sound is given (as is the case in every report from mills in Howe Sound).
3. No data on crab gonads were reported.

Differences Observed:

Exposure-zone mussels were generally smaller and lighter but had slightly higher condition factors (not tested statistically).

Dungeness crabs from exposed and reference areas had similar mean carapace width and body weight. Mean hepatopancreas weight and mean hepatosomatic indices were higher in the exposed zone (but not statistically tested).

EEM Cycle 2 Considerations:

An alternate reference area should be considered so that finfish (slender sole) can be obtained for the Cycle 2 AFS. If blue mussels are to be used as the second sentinel species, they should be compared using ANOVA and ANCOVA tests of growth, energy storage and reproductive parameters. An alternative may be required if local referrence site is not acceptable.

Mill: Weyerhaeuser Ltd. Canada, Kamloops, BC

Problems:
The effluent mixes rapidly and was estimated (by measuring dissolved sodium and tannin/lignin concentrations) to be at less than 1% concentration in the water column within 60 m downstream of the diffuser. Fish collected in the exposure zone are therefore not exposed to 1% effluent. Tracers in fish tissue (RFAs and dioxins/furans) did not confirm exposure/non-exposure of fish; however, sediments from the exposure area contained elevated levels of DHA and guiacols relative to reference-area sediments.

Differences Observed:
Reference fish were older for both species and sexes.
Weight at age was greater in reference fish of both species and sexes. Male and female largescale sucker were also longer at age in the reference zone. Reference male largescale suckers were heavier (at body length) than exposed male suckers, but reference peamouth chub were lighter (at body length; both sexes) than exposed chub.
Exposed female chub had greater LSI values (heavier liver at body weight) than reference female chub.
Gonad weight (at body weight) showed significant interaction in female peamouth chub.

EEM Cycle 2 Considerations:
There is a limited exposure zone for this study, and it includes both riverine and lacustrine habitats.

Mill: Williston Reservoir - 2 Mills (Finlay Forest Industries and Fletcher Challenge Canada Mackenzie Pulp, both in Mackenzie, BC)

Problems:

1. Too few fish; only one sentinel species in sufficient number (lake whitefish).
2. Poor reporting of statistics.

Differences Observed:
According to bar graphs in text, may not be statistically significant: Graphs show little difference in condition factor for either species among all 3 sites. LWF females seem to have higher mean GSI values in the reference than in NF (for ages 3 and 4 years; most of the sample) but SDs overlap. LSS females GSI differ with age class. LSIs are generally higher in NF than in reference.

EEM Cycle 2 Considerations:
More effort may be required to capture enough largescale sucker.

Table 20 summarizes the data on each mill and the details of the Fish Survey as provided in the Cycle One report. Mills are listed in alphabetical order. "Differences in exposed relative to reference fish" are for statistically significant differences only, unless otherwise noted.

Table 20. Summary of Fish Survey designs and results for Pacific and Yukon region mills.
Page 1

Mill & Location	**Type of Mill**	**Discharge environment**	**Receiver flow**	**Effluent flow**	**Approx. effluent conc.**	**Sentinel species**	**Collection method, dates**	**Numbers collected**	**Differences in exposed fish relative to reference**	**Problems**
Avenor Inc., Gold River BC	Kraft, 664 ADt/d, 50-70% ClO_2 sub	2^0 trt (AST) discharge to fjord	19.3 m^3/s (Gold R., low flow)	90,900 m3/d	>1% at ~1 km up and down of diffuser, all across channel	widow rockfish (*Sebastes entomelas*)	gillnets (1.5-2.5" mesh) overnight and crab. traps,divers 6-15 July 1995	11 M, 11 F ref; 51 M, 34 F exp.	-no mention of age because the age data was considered unreliable -exposed males heavier livers (x length)	-municipal discharge and fish hatchery discharge to exposure site - only 1 sentinel sp -undeveloped gonads (timing) -dioxin/furan tracer higher in ref fish -fish not aged -ref fish all smaller
Celgar Pulp Company, Castlegar, BC	1068-1275 ADt/d; bleached kraft (100% ClO_2)	2^0 trt (AST) Columbia R.	588-708 m^3/s during sampling; 142 m^3/s low flow	119,000 m^3/d	0.54-1.03% NF by Na tracer analysis	mountain whitefish (*Prosopium williamsoni*)	mainly elctroshock, some gillnetting (monofilament, 51-76 mm) and seining	22 M, 21 F ref; 17 M, 17 F exp.	F: -age, +condition, +length, +egg volume M: -age, -condition, +gonad weight	-immature fish included in statistical analyses?
						longnose sucker (*Catostomus catostomus*)	10-17 Oct., 1994.	21 M, 20 F exp.	no stats	

Table 20. Summary of Fish Survey designs and results for Pacific and Yukon region mills. (Cont'd)
Page 2

Mill & Location	Type of Mill	Discharge environ-ment	Receiver flow	Effluent flow	Approx. effluent conc.	Sentinel species	Collection method, dates	Numbers collected	Differences in exposed fish relative to reference	Problems
Crestbrook Forest Industries Ltd., Cranbrook BC	671-782 ADt/d; bleached kraft mill 100% ClO_2	ASB, 3^0 trt (colour ciarifier) then discharge to Kootenay R.	not reported	41,800 - 47,800 m^3/d	<1% at 50 km in lowest flows; o/w <1% before Skook-umchuk	mountain whitefish (*Prosopium williamsoni*)	electro-shocking; 19-25 Oct. 1994.	21 M, 21 F ref; 21 M, 21 F exp.	F: length SI, -age, -gonad weight (when gravid females removed), M: -age, -gonad weight	-small sample sizes for largescale sucker -most fish were rated 7 for maturity, *i.e.* "spent or recovering" gonad condition; only 5 females had eggs
						large-scale sucker (*Catostomus macrocheilus*)		7 M, 13 F ref; 8 M, 19 F exp.	too few for stats; plots indicate fish are older and males heavier, greater GSI in exp.	-exp/non-exp confirmed for LSS only; MWF move too much?
Crofton Pulp and Paper (FCC), Crofton BC	900 ATD/d kraft mill, 75-100% ClO_2 sub	UNOX trt then discharge to coast	not reported	141,000 m^3/d	1% at 600 m	English sole (*Porophrys vetulus*)	trawling, longlines, cod traps; March 21-26, 1995	15 M, 28 F ref; 11 M, 15 F NF.	only 1 ref fish, 2 NF fish were at advanced state of maturity; cond factor higher in ref; HAI mean higher in NF (but no stats so may not be significant)	-too few sole from ref and NF sites -graphs for comparison but no stats or power analysis
						Pacific oyster (*Crasso-strea gigas*)	by hand at low tide	21 oysters at each site (NF, FF, ref)	most abundant at NF (qualitative); heaviest and longest in NF, higher condition in ref, but no stats so may not be significant	-poor timing for fish and oyster collection? (un-developed gonads) -no crabs from ref
						Dungeness crab (*Cancer magister*)	crab traps, overnight; Mar. 1995	0 ref; 32 M, 1 F, NF; 54 M, 1 F, FF	used for organochlorine analyses of hepatopancreas only	so no comparison of contam. levels among sites

Table 20 Summary of Fish Survey designs and results for Pacific and Yukon region mills. (Cont'd)
Page 3

Mill & Location	**Type of Mill**	**Discharge environ-ment**	**Receiver flow**	**Effluent flow**	**Approx. effluent conc.**	**Sentinel species**	**Collection method, dates**	**Numbers collected**	**Differences in exposed fish relative to reference**	**Problems**
Crown Packaging Ltd., Burnaby BC (Paperboard)	paper recycling mill, unbleached; 500 ADt/d	ASB-treated then to North Arm of Fraser R.	900-10,000 m/s in summer	5,900-10,000 m^3/d	1% at 325 m down and 430 m up; 0.2% at 400m down, 1230 m up	prickly sculpin (*Cottus asper*)	beach seine (6-19 mm mesh) and Gee-type minnow traps	19 M, 19 F, 3 im ref; 16 M, 20 F, 3 im exp.	weight (x age) differed for male and female prickly sculpins	-2 mills and a metal refinishing plant discharge within 3 km upstream; ref site chosen to measure impact of Crown mill only
						Pacific staghorn sculpin (*Leptocottus armatus*)	16 Nov. - 15 Dec. 1994	10 M, 11 F ref; 10 M, 14 F exp.	gonad wt (x lgth) differed for female staghorns	-flounder were all juvenile; gonads small and without eggs in all
						starry flounder (*Platichthys stellatus*)		33 ref, 33 exp; all juveniles so not sexed	no significant differences	fish species examined -no summary of age data; body wt and lgth were used as a measurement of age but regressions do not indicate strong correlation--why used?
Elk Falls P&P (FCC), Campbell River, BC	2,100 Adt/y kraft pulp; up to 100% ClO_2 sub	UNOX then discharge to Discovery Passage	not reported	198,000 m^3/d	<1% beyond 500 m from diffuser	Dungeness crab (*Cancer magister*)	crab traps (overnight), 8-22 April 1995.	41 M, 2 F, NF; 106 M, 36 F, FF; 37 M, 1 F, ref.	larger hepato in NF; corr. analysis shows exp site has a weak association with gill fouling and moderate numbers of lesions	-too few rockfish and most had undeveloped gonads -no gonad stats for crabs?
						quillback rockfish (*Sebastes maliger*)	handlines, longlines and cod traps (12 hrs)	28 M, 14 F exp; 12 M, 10 F ref.	exp males younger, males different age distributions females higher wt (x lgth) in exp	

Table 20 Summary of Fish Survey designs and results for Pacific and Yukon region mills. (Cont'd)
Page 4

Mill & Location	**Type of Mill**	**Discharge environment**	**Receiver flow**	**Effluent flow**	**Approx. effluent conc.**	**Sentinel species**	**Collection method, dates**	**Numbers collected**	**Differences in exposed fish relative to reference**	**Problems**
Eurocan Pulp and Paper Ltd., Kitimat, BC	1,121 Adt/d in 1995; unbleached kraft/CMF paper and linerboard	2^0 trt (ASB) then by diffuser to Kitimat R., hear mouth	129 m^3/s mean annual flow	65,000	10% in ~500m, 3% at mouth, 1% in upper estuary, all at low flow	starry flounder (*Platichthys stellatus*)	seine nets, by boat and person, and minnow traps	30-76 used from ref1; 35-140 used from exp; none from ref2	sexes pooled for analysis: ref1 fish slightly heavier (ANOVA: p=0.03), condition, liverwt, age-at-wt, age all sig different (exp and re1 only); fish too small for gonad measurement (mean ages 0.8- 1.1 years)	-Alcan, Methanex, STP and DFO hatchery discharge upstream -too few fish from some sites -ref2 and exp sites may be too close;
						prickly sculpin (*Cottus asper*)	November, 1994	31 M, 7 F ref 1; 43 M, 20 F ref 2; 20 M, 21 F exp.	condition differed for males, liver weight for females; SI for gonad wt, age-at-wt for males, females & pooled	fish moving between? RFAs suggest ref2 fish are exposed
						staghorn sculpin (*Leptocottus armatus*)		10 M ref 1; 4 M, 9 F exp; none from ref2	condition, liver wt, age-at-wt differed significantly between sites for pooled sexes, exp and ref1 only (no fish from ref2)	
Fibreco Pulp, Taylor BC	BCTMP mill; 500 ADMt/d pulp with H_2O_2 brightening	2^0 trt AST then to Peace River	685 m^3/s mean for Aug. 1995; 1,110 m^3/s annual mean 1995	13,000 m^3/d	<1% and likely <0.1% in NF and FF	mountain whitefish (*Prosopium williamsoni*)	electro-shocking; Aug. 1995	20 M, 20 F at each of the three sites (NF, FF, ref)	Females: NF oldest, FF heaviest (x age), FF longest (x age), ref heaviest (x lgth), SI for liver wt (x body wt); NF males had lowest liver wt (x body wt)	-GSI, LSI listed in contents but not included? also numbers of fish per site? list of total catch at each site?
						longnose sucker (*Catostomus catostomus*)		20 M, 20 F at each of the three sites (NF, FF, ref)	Females: ref oldest, NF longest (x age), ref heaviest (x lgth), liver wt (x body wt) lowest in FF, equal in ref & NF	

Table 20. Summary of Fish Survey designs and results for Pacific and Yukon region mills. (Cont'd)
Page 5

Mill & Location	**Type of Mill**	**Discharge environment**	**Receiver flow**	**Effluent flow**	**Approx. effluent conc.**	**Sentinel species**	**Collection method, dates**	**Numbers collected**	**Differences in exposed fish relative to reference**	**Problems**
Harmac Pacific Inc., Nanaimo, BC	bleached kraft pulp mill, 100% ClO_2 sub; prod. 1180 ADt/y	2^0 (AST) then discharge to coast	not reported	160,000 m^3/d	1.5-0.5% in 100m from outfall	English sole (*Parophrys vetulus*)	trawling (at 40-160 m depth), steel crab traps, nylon prawn traps	21 M, 24 F ref; 41 m, 20 F NF; 27 M, 24 F FF	females heavier (x lgth) in NF and FF than ref, sig diff liver wt (x body wt), FF older (outliers removed) males heavier (x lgth) in NF and FF than ref, older in FF, NF than ref	-ANOVAs on length and weight of both spp used combined male and female data -cannot interpret their "power chart"
						dungeness crab (*Cancer magister*)	October 1995	36 M, 4 F ref; 23 M, 17 F NF; 35 M, 5 F FF	females lighter (x lgth) in NF than in ref and FF exposed males smaller gonads (x body wt)	and their ANCOVA printouts are out of order therefore hard to interpret -'problems' with fish sex. maturity?- tracer unsuccessful
MacMillan Bloedel, Alberni Pulp and Paper Mill Port Alberni, BC	groundwood, CTMP, peroxide bleaching, 900 Adt/y newsprint	2° trt (AST) then release to fjord (Alberni Inlet);	45 m^3/s (Somass R. low flow)	106,000 m^3/d	>50% at outfall, soon mixed to >1%	English sole (*Parophrys vetulus*)	otter trawl at 10-100 m, late Aug.- early Sept.	40 exp; 1 ref1; 0 ref 2; 11 ref2 (Barkley Sound)	no stats	-report incomplete: data summaries and analyses missing -too few sole; try sculpin or eelpout instead?
	and light-weight specialties					threespine stickleback (*Gasterosteus aculeatus*)	beach seine (0.5 mm mesh) and Gee traps, in May (before moved to freshwater to spawn)	145 exp; 133 ref1; 263 ref2		-plots should be designed to compare exp and ref fish of one sex; not males and females from same site.

Table 20. Summary of Fish Survey designs and results for Pacific and Yukon region mills. (Cont'd)
Page 6

Mill & Location	**Type of Mill**	**Discharge environment**	**Receiver flow**	**Effluent flow**	**Approx. effluent conc.**	**Sentinel species**	**Collection method, dates**	**Numbers collected**	**Differences in exposed fish relative to reference**	**Problems**
Macmillan Bloedel, Powell River, BC	1534 ADt/d of kraft and CTM pulp; 80-95% ClO_2 sub	2° trt (UNOX) then discharge to Malaspina Strait	not reported	194,000 m^3/d	1% 3Km	English sole *Parophrys vetulus)*	trawl, longline and cod traps; March (exp) and May (ref) 1995	13 M, 16 F ref; 1 M, 1 F exp.	no stats; undeveloped gonads	-why not use slender sole? -timing of collection -no stats for sole
						Pacific oyster (*Crassostrea gigas)*	hand picked, timing as above	21 oysters from each of 4 NF, 2 FF and 2 ref sites	no stats but lower condition, greater size in exposeure (timing of collection?)	
						Dunge-ness Crab (*Cancer magister*)	crab traps, timing as above	23 NF, 10 FF, 2 ref		
Repap BC Inc. (Skeena), Prince Rupert, BC	1,300 ADt/d kraft pulp; 40-100% ClO_2 sub	Unox then discharge to Porpoise Harbour	not reported	159,000 m^3/d	not reported	Rock sole (*Lepidop-setta bilineata*)	trawl, longlines, cod traps; April 28-May 8, 1995	7 M, 7 F ref; 7 M, 5 F exp.	too few for stats	-too few rock sole -what about the 150 blue mussels collected? -why no figures for crab comparisons?
						Dunge-ness crab (*Cancer magister)*	crab traps, overnight	55 M, 32 F ref; 73 M, 5 F NF; 119 M, 33 F FF.	results state no differences among sites (p.4/36); then CA shows NF crabs are heavier in relation to carapace width and have heavier hepatopancreas than reference crabs.	-why such differenct numbers of inverts from ref and exp trawls? habitat differences?

Table 20. Summary of Fish Survey designs and results for Pacific and Yukon region mills. (Cont'd)
Page 7

Mill & Location	Type of Mill	Discharge environment	Receiver flow	Effluent flow	Approx. effluent conc.	Sentinel species	Collection method, dates	Numbers collected	Differences in exposed fish relative to reference	Problems
Scott Paper Ltd., New Westminster BC	292 ADmt/d tissue; groundwood mill on-site plus purchased pulp	dissolved air effluent clarifiers then to North Arm of Fraser R.	~1400 m^3/s at Hope, BC (low flow)	15,200 m^3/d	>1% at 1 km up and 200 m down, low flow	starry flounder (*Platichthys stellatus*)	beach seining, G traps; Oct. 1994	97 ref; 53 NF; 69 FF	ANOVAs on juveniles, sex unknown, ref sites combined: sig interaxn for body wt (x lgth), no other differences.	-confounding discharges, up and downstream (b/c of tidal action); also dredging - ref site too close to exp?
						prickly sculpin (*Cottus asper*)		14 M, 6 F, 48 im ref; 15 M, 2 F, 39 im NF; 4 M, 7 F, 29 im FF.	body wt (x lgth) differed for juveniles; female liver wt (x body wt) differed; juvenile gonad wt (x body wt) showed SI	-too many immatures, too few adults -exp sites combined for analysis?
Upper Fraser River - 4 Mills; Prince George and Quesnel, BC	CTMP, kraft, 100% ClO_2 4800 Adt/d combined production	Fraser R.	97 to 272 m^3/s	10,000 to 150,000 m^3/d per mill	~2% at diffuser; <1% between Pr George and Quesnel and down of Ques.	Large scale sucker (*Catostomus macrocheilus*)	shocking, gillnets (51-76 mm, monofilament), beach seine (6.3mm) and angling	24 M, 23 F ref; 24 M, 22 F PG; 25 M, 23 F WP; 22 M, 20 F Qu	ref and PG females older than WP and Qu; size (x age) SI, gonad wt (x body wt) SI; liver wt (x body wt) highest at PG; LSI highest at Qu, lowest at ref but not signif. males largest (x age) at PG, smallest at Qu; gonad wt (x body wt) SI.	-most fish of both sentinel spp. had undeveloped gonads -no tracer in fish? (Na tracer in water was successful)
						peamouth chub (*Mylacheilus caurinus*)	20 Sept - 4 Oct., 1994	25 M, 27 F ref; 20 M, 20 F PG; 20 M, 27 F WP; 12 M, 14 F Qu.	females older at ref and PG than WP and Qu; longest (x age) at PG and WP; heaviest gonads (x body wt) at ref and PG males longest (x age) at PG, shortest at ref and Qu; gonad wt (x body wt) SI (higher slope at ref)	-only 3 females with eggs at Qu

Table 20. Summary of Fish Survey designs and results for Pacific and Yukon region mills. (Cont'd)
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Mill & Location	**Type of Mill**	**Discharge environment**	**Receiver flow**	**Effluent flow**	**Approx. effluent conc.**	**Sentinel species**	**Collection method, dates**	**Numbers collected**	**Differences in exposed fish relative to reference**	**Problems**
Western Pulp Partnership and Howe Sound Pulp and Paper	bleached kraft pulp 60-100% ClO_2 sub; 1550 ADt/d (HSPP) 700 Adt/d (WPP)	UNOX then discharge to Howe Sound (fjord with Squamish River input)	not reported	80,100 m^3/d (HSPP) 66,700 m^3/d (WPP)	not reported	Blue mussel (*Mytilus edulis*)	hand picked "mussels of varying sizes" at low tide; March 1995 in exposure zone, May 1995 in ref	70 NF1; 40 ref1; 88 NF2; 60 ref2	no stats but appear smaller in exp (earlier, colder time of collection?); condition indices higher in ref (even with smaller exp mussels removed)	-NF1=WPLP outfall, ref1=upper Toba inlet -NF2=HSP&P outfall, ref2=lower Toba Inlet
						Dungeness crab (*Cancer magister*)	crab traps overnight; timing as above	42 M, 7 F NF1; 42 M, 13 F NF2; 4 M ref1; 31 M, 6 F ref2	no stats; mean hepatopancreas as % of body wt is higher in NF than ref; SDs overlap	-all exp samples collected in March, all ref samples in May -some exp mussels too small to
						English sole (*Parophrys vetulus*)	trawl, longlines, cod traps; timing as above	1 M ref; 1M, 1F exp (WPLP)		measure dry wt, and therefore condition indices
WPLP, Port Alice Operations, Port Alice, BC	sulphite mill, 470 ADt/d, chlorine bleached	2° trt (ASS) began **after** EEM study; discharge to Neroutsos Inlet (fjord)	not reported	13,7000 m^3/d	<1% throughout Neroutsos Inlet	slender sole (*Lyopsetta exilis*)	longline, angling, trawl, cod traps; 10-16 May, 1995	20 M, 20 F exp; 0 from ref	from one site only; no stats	-too few slender sole -longlines and cod traps in exp only -why no ANOVAs on blue mussel
						blue mussels (*Mytilus edulis*)	hand picked	63 ref; 63 exp.	-exposed mussels were generally smaller and lighter but had slightly higer cond. factor; not statistically tested	data not included
						Dungeness crab (*Cancer magister*)	crab trap overnight	23 M, 20 F ref; 12 M, 27 F exp.		

Table 20. Summary of Fish Survey designs and results for Pacific and Yukon region mills. (Cont'd)
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Mill & Location	**Type of Mill**	**Discharge environ-ment**	**Receiver flow**	**Effluent flow**	**Approx. effluent conc.**	**Sentinel species**	**Collection method, dates**	**Numbers collected**	**Differences in exposed fish relative to reference**	**Problems**
Weyerhaeuser Canada Ltd., Kamloops BC	kraft, 100% ClO_2 1250 ADt/d	2^0 trt (ASB) Thompson R.	0.2-0.5 m/s NF; zero in FF and ref	139,000 m^3/d	>1% at nearly 4 km down of diffuser at lowest receiver flows	large-scale sucker (*Catostomus macrocheilus*)	electro-shock, gill nets (51-76 mm mesh), angling and set lines	22 M, 21 F NF; 21 M, 21 F ref	F: -age, -length, -body weight M: -length, -body weight, -condition	-tracers do not confirm exposure/non-exposure of fish; dissolved Na shows that the plume is <1% at NF and FF exp sites and 0% at ref sites
						pea-mouth chub (*Mylocheilus caurinus*)	20 Sept-20 Oct 1995.	17 M, 30 F NF; 21 M, 21 F ref	exposed fish younger, lighter (x age) but heavier (x lgth); exp females larger liver (x body wt), sig interaxn for gonad wt (x body wt)	-no info on mill processes or effluent treatment -different capture methods leading to different size fish?
Williston Reservoir - 2 Mills, Mackenzie BC	FCC: Kraft softwood pulpmill (610 Adt/d) FFI: ground-wood RMP and paper mill (~500 ADt/d) H_2O_2 brightening	2^0 trt (ASB) then diffuser to reservoir	not reported	FCC: ~51500 m^3/d; FFI: ~12600 m^3/d	not reported	large-scale sucker (*Catostomus macrocheilus*)	gillnets (60,76 and 91 mm mesh), set in morning;	27 M, 10 F ref; 19 M, 13 F NF; 2 M, 2 F FF (Sept/Oct 1994 only)		
						lake whitefish (*Coregonus clupeaformis*)	Sept/Oct 1994 and March 1995	23 M, 22 F ref; 19 M, 22 f NF; 9 M, 25 F FF (Sept/Oct 1994 only)		

APPENDIX IV

Regional Synthesis of Sentinel Species and Problems for Mills Demonstrating Various Degrees of Capture Success in Cycle One

prepared by

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Appendix IV

a) Pacific/Yukon

Status	Mill	Receiving Environment	Species	Problems
Successful	Canfor/Northland	?	Largescale sucker Peamouth chub	?
	Fibreco	Riverine	Mountain whitefish Longnose sucker	none identifed
Partially	Weyerhaueser	Riverine	Largescale sucker Peamouth chub	small plume; unknown exposure; different habitats downstream
	Alberni	Estuarine	Threespine stickleback English sole	unconfirmed exposure; and undeveloped gonads
	Cariboo/Quesnel	Riverine	Largescale sucker	no tracer; undeveloped gonads
	Crestbrook	Riverine	Mountain whitefish	gonads spent
	Eurocan	?	Prickly sculpin	? high numbers of starry flounder (juveniles)
	Harmac	Marine	English sole	unconfirmed exposure; undeveloped gonads
	Paperboard	?	Prickly sculpin	? high numbers of starry flounder (juveniles)
	Finlay/Mackenzie	Lacustrine	Lake whitefish	poor report (difficult to evaluate)
Other	Scott	?		high numbers of starry flounder (juveniles) and prickly sculpin (juveniles)

b) Prairie/Northern

Status	Mill	Receiving Environment	Species	Problems
Partially	ANC Whitecourt	?	Lake chub Longnose sucker	?
	Pine Falls	Riverine	Whitesucker Shorthead redhorse	unconfirmed exposure
	Weldwood	Riverine	Longnose sucker Mountain whitefish	fish migrate; reference on separate river system
	Millar Western	Riverine	Lake chub Longnose sucker	no tracer; no aging
	Grande Prairie		Mountain whitefish Longnose sucker	reference on separate river system; no stats
	Repap Le Pas	?	White sucker	?

c) Ontario

Status	Mill	Receiving Environment	Species	Problems
Successful	Sonoco	Riverine	Silver redhorse Shorthead redhorse	none identified
	Strathcona	?	Smallmouth bass Rock bass	?
Partially	Iroquous Falls	?	Goldeye Walleye	?
	KC Terrace Bay	Lacustrine	White sucker Longnose sucker	unconfirmed exposure
	Domtar Trenton	Riverine	Carp Silver redhorse	needed different reference areas for each species; evidence of river continuum response
	Domtar Red Rock	Lacustrine	White sucker Longnose sucker	gonads weights in exposure higher lower or no different than reference (depends on species and whether weight or length used as covariate)
	4 mills St. Cath.	Riverine	Shorthead redhorse	confounding influences; small gonads (sampling possible only in early August)
	Stone Kenora		White sucker	habitat differences
	Avenor TB	Riverine	White sucker	confounding dischargers; unconfirmed exposure; pooled lacustrine and riverine exposure data
	Provincial Papers	Lacustrine	Spottail shiner	fish too young; habitat differed; unconfirmed exposure; confounding factors
	St. Marys Paper	Riverine	White sucker	rapid dilution; confounding dischargers; habitat differences

	Abitibi TB	Lacustrine	Spottail shiner	fish too young
	Beaver Thorold		Pumpkinseed	different size range in reference and exposure
Marginally	Avenor Dryden	Riverine	Walleye	fishing difficult; some fish purchased
	Spruce Falls Kap.	Riverine	White sucker	habitats differed
	James River Mar.	Lacustrine	Longnose sucker	unconfirmed exposure
	Domtar Cornwall		Yellow perch	rapid dilution; confounding dischargers; habitat differences; different size range in reference and exposure
	Mallette SR		White sucker	unconfirmed exposure
Other	Sturgeon Falls			high numbers of spottail shiner (not sexed); different habitat features

d) Quebec

Status	Mill	Receiving Environment	Species	Problems
Successful	Maclaren Thurso	Riverine	Brown bullhead Yellow perch	undeveloped gonads; unconfirmed exposure
	J.Ford Portneuf	Riverine	White sucker Longnose sucker	undeveloped gonads
Partially	Donohue Amos	Riverine	Longnose sucker Brown bullhead	problems aging; unconfirmed exposure; no barrier between reference and exposure (close together)
	Donohue St. Fel.	Riverine	Brown bullhead	confounding discharges; hydro regulation of river; male gonads undeveloped
	Alliance Donn.	Lacustrine	Longnose sucker	confounding factors; male gonads undeveloped
	Rolland St-Jerome	Riverine	White sucker	none identified
	Scott Lennoxville	Riverine	White sucker	unconfirmed exposure; reference and exposure close together
	Domtar Windsor	Riverine	White sucker	male gonads undeveloped; multiple dischargers upstream; habitat differences
	Maclaren Masson	?	Yellow perch	?
Marginally	Cascades Kingsey	Riverine	Silver redhorse	none identified (report incomplete)
	Cascades Est Ang.	Riverine	White sucker	?
	Cartons La Tuque	Riverine	White sucker	confounding factors

	Scott Crabtree	Riverine	White sucker	none identified
	Cascades Joliette	Riverine	White sucker	report incomplete
Other	Perkins Lachute	Riverine		no adult fish (all immature)
	Abitibi Alma	Riverine		no adult fish (all immature)
	CascadesBreakey.	Riverine		habitat unsuitable for AFS
	FF. Soucy R-du-L	Riverine		no fish in exposure

e) Atlantic

Status	Mill	Receiving Environement	Species	Problems
Partially	ABT Canexel	Estuarine	Winter flounder	undeveloped gonads; small
			Longhorn sculpin	effluent plume; unconfirmed effluent exposure
	Corner Brook	Estuarine	Winter flounder Cunner	unconfirmed exposure
	St-Anne Nackawic	Riverine	White sucker	habitat differences; unconfirmed exposure
	Bowater Mersey	?	Longhorn sculpin	?
	Minas Basin		Tomcod	no exposure at low tide; small effluent plume (disperses rapidly) high numbers of Atlantic silverside (immature)
Marginally	Repap Kraft	Estuarine	Tomcod	none identified
	Fraser	?	Yellow perch	?
	Abitibi Grand Falls	?	Threespine stickleback	?
	Abitibi Stephenville	Estuarine	Winter flounder	undeveloped gonads
Other	Abitibi Grand Falls	Riverine		lack of diverse fish population (3-spined stickleback)